THE ANALYST

Editorial

As the current trends and aspects of chemical analysis change and develop, so too must the content of *The Analyst* alter in character if our journal is to fulfil its rôle and keep analysts throughout the world informed of all that is latest in their profession and of what is passing through the minds of those in the forefront of progress.

Perhaps the chief step that we have taken to this end in the past few years has been the commissioning of review articles designed to give a condensed account of a new subject, or a rational statement of the position of one that is not so new, and thereby to provide the analyst unfamiliar with a particular portion of the whole subject with enough information to allow him to undertake work in it.

These review articles together with first-hand accounts of all that is new in analytical chemistry have hitherto provided most of the material we have published, and we shall continue to look to such sources for the bulk of the contents of each issue of the journal, bearing continually in mind that *The Analyst* must cover the whole vast range of the subject of analytical chemistry, and continuing to invite contributors to submit papers dealing with any part of it.

However, it has become apparent that much material is produced each year that does not come within the recent ambit of the journal. Of this a good deal is ephemeral and is probably better published in a weekly paper, but some is of lasting importance and deserves to be included in a journal that is more widely preserved.

In this category we may place pronouncements emanating from the pens of leaders in our profession. Hitherto we have been very selective in dealing with these. It is now intended to extend this privilege somewhat so as to include accounts of lectures of general outstanding interest; and in this issue we present in this way the full text of the lecture given last October by Mr. H. N. Wilson to the North of England Section on "The Changing Aspect of Chemical Analysis." The lecture needs no commendation from us; it speaks for itself, and must surely stimulate all who read it.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

THE ROYAL SOCIETY OF LONDON

On Monday, July 18th, 1960, in the Rooms of the Royal Society, Burlington House, London, the President, Mr. R. C. Chirnside, presented the following Address to the Royal Society on the occasion of the celebration of the tercentenary of its foundation—

AN ADDRESS TO THE PRESIDENT, COUNCIL AND FELLOWS OF THE ROYAL SOCIETY OF LONDON

The President, Council and Members of the Society for Analytical Chemistry offer their sincere and hearty congratulations to the Royal Society on the occasion of the tercentenary of its foundation.

The Society for Analytical Chemistry welcomes the opportunity to be associated with other Learned Societies and Scientific Institutions in paying tribute to the Royal Society of London which takes precedence as the parent of all our Scientific Societies. Throughout the three centuries of its existence it has upheld the prestige of Science and maintained and extended the objects of its founders to the benefit of civilisation at large.

The Society for Analytical Chemistry recalls with gratitude that the Royal Society has fostered the progress of learning and research in Chemistry no less than in other branches of Science. It is their earnest hope that the Royal Society supported and fortified by its great traditions may continue its work with undiminished vigour and success.

Given under the Seal of the Society the 15th day of July, 1960

(*Signed*) R. C. Chirnside (*President*)
A. J. Amos (*Honorary Treasurer*)
R. E. Stuckey (*Honorary Secretary*)

Seal of The Society for Analytical Chemistry

DEATHS

We record with regret the deaths of

Arthur Sidney Carlos
Lewis Goudin Spire Hebbs
James Herbert Oliver.

NORTH OF ENGLAND SECTION

The twenty-third Summer Meeting of the Section was held at the Imperial Hotel, Llandudno, from Friday, June 17th, to Monday, June 20th, 1960.

The Chairman of the Section, Dr. J. R. Edisbury, presided over an Ordinary Meeting at 10.15 a.m. on Saturday, June, 18th, at which J. B. M. Coppock, O.B.E., Ph.D., F.R.I.C., gave a lecture entitled "The Baking Scientist."

On the Saturday evening, the party saw the show at the Arcadia Theatre, Llandudno, and on the Sunday afternoon made a coach tour, taking tea at Beaumaris.

WESTERN SECTION

A joint Summer Meeting of the Section with the South Western Counties Section of the Royal Institute of Chemistry was held in Plymouth on Friday and Saturday, May 27th and 28th, 1960.

At 6.30 p.m. on Friday, May 27th, in the Technical College, Tavistock Road, Plymouth, L. H. N. Cooper, Ph.D., D.Sc., gave a talk on "Trace Elements in Sea-water." The Chair was taken by the Chairman of the Western Section, Dr. G. V. James, M.B.E., F.R.I.C.

By kind permission of the Director, Dr. F. S. Russell, C.B.E., F.R.S., a visit was paid to the Marine Biological Laboratory, Citadel Hill, Plymouth, at 10.15 a.m. on Saturday, May 28th.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held on Thursday, May 26th, 1960, when 21 members of the Group visited the Beecham Research Laboratories at Brockham Park.

Tours of the laboratories were made during the morning and afternoon, and the thanks of the participants were conveyed by Dr. J. I. M. Jones, F.R.I.C., Chairman of the Group.

Obituary

GEORGE TAYLOR

GEORGE TAYLOR, Past President of the Society, died at his home in Hampstead on February 28th, 1960, his seventy-ninth birthday.

Taylor started his scientific career as an Assistant to Thomas Pooley, who was Public Analyst for the County of Essex, whilst continuing his studies as a part-time student at Finsbury Technical College. After Pooley's death in 1905, he became an Assistant to Bernard Dyer. Taylor passed the fellowship examination of the (Royal) Institute of Chemistry under the old regulations in 1917 and subsequently, in the early twenties, he was appointed Joint Public Analyst and Deputy Official Agricultural Analyst for a number of counties. When, in 1926, Bernard Dyer converted his practice into a partnership, Taylor became one of the Partners. In 1948, on the death of Bernard Dyer, he was appointed Official Agricultural Analyst for the counties for which he had previously been Deputy.

From his earliest days Taylor took a great interest in the affairs of the Society and was Honorary Treasurer from 1940 to 1949. In this capacity he covered the difficult war years, but in spite of the many problems that faced the officers during those years, he was able to save quite considerable sums out of the Society's limited income. He was President of the Society in 1949 and 1950.

Taylor had a great love of Committee work and, apart from serving on the Publication Committee, the Analytical Methods Committee and several of its Sub-Committees, he represented the Society on a number of important Government Committees, such as the Food Standards Committee and the Agricultural Advisory Committee of the Ministry of Agriculture, Fisheries and Food. For his work on the former Committee he received the O.B.E. He was also the Society's representative on the Parliamentary and Scientific Committee. Taylor's term of presidency of the Society coincided with the period of intense activity over the change of name of the Society. He played a leading part in the formation of the Association of Public Analysts, realising the ever-growing necessity for a separate organisation that could speak with one voice for Public Analysts. As the Association's first president he had the difficult task of knitting together the many vigorous and individualistic personalities that made up the Association's first Council, and there is little doubt that the respect and regard with which the Association is held to-day is in no small way due to his wisdom and guidance in this critical year of inauguration.

He was an examiner in Branch E for 4 years, and there are many chemists in the country to-day who will remember with gratitude his very human approach to examinations. He served successively as a Member of Council, as a Vice-President and as a Censor. In 1947 he gave the Streatfeild Memorial Lecture.

He was an enthusiastic hockey player and continued playing up to the age of fifty. Tennis and bridge were among his other activities outside the laboratory, but analytical chemistry remained his principal interest in life until the end. Throughout his life he had an avid interest in new analytical methods and techniques, and was not satisfied with reading about them in books and journals or with merely seeing the results obtained by assistants, but was always eager to try then out himself. Indeed, nearly every day of his working life he devoted some part of his time to working at the bench.

Taylor's outstanding characteristics were complete integrity and common sense. He was always willing to give advice or assistance and was approachable at any time. He was one of the few people in this world who thought well of all men, and his wisdom and charm will be missed by all.

J. H. HAMENCE

The Changing Aspect of Chemical Analysis*

By H. N. WILSON

(*Imperial Chemical Industries Ltd., Billingham Division, Billingham, Co. Durham*)

AFTER the visit to the analytical laboratories in the Research Department at the Billingham Division of Imperial Chemical Industries Ltd. earlier to-day, it seems to me that a general type of lecture will be more appropriate than the discussion of a particular topic. I therefore propose to review some of the present tendencies in analytical chemistry, because it is a useful exercise to consider the broad field, rather than the individual plants. This will not enable us to forecast what is going to happen next (if I had given this lecture 8 years ago I could not have mentioned gas chromatography, as it was in 1952 that it burst upon an astonished world), but it may help us to decide which of the plants are most worth cultivating. I am going to talk from the point of view of an industrial analyst, most of whose time is nowadays given to administration, and I propose to rely very largely on my own experience, rather than to make an attempt to review everything. This will leave some gaps, but ought to ensure that the picture is fairly realistic.

I would like to emphasise that I am going to talk about *chemical analysis* and not *analytical chemistry*. The difference is not always sufficiently appreciated, particularly in academic circles, where they teach *analytical chemistry*, and often restrict it to *inorganic* chemistry, not having yet caught up to the fact that organic analytical chemistry is—in industry, in agriculture and, I expect, in public analysts' laboratories—equally important. But in carrying out chemical analysis nowadays we are half the time not primarily concerned with chemistry, but with physics. My Company produced a book on analytical techniques, which has had a certain amount of success. We called it "Analytical Chemistry—The Working Tools," because it was about the tools of the trade. But one reviewer rather acidly pointed out that this title was a misnomer, as there was hardly any chemistry in it, it was mostly about physics. Nevertheless, we are not analytical physicists, but analytical chemists, because what we are interested in is not usually the physical state of our samples, but the chemical composition, and we use any criterion—chemical, physical or biological—that will help us to obtain the desired information. This leads me to attempt a definition of chemical analysis. "Chemical analysis is a body of techniques, chemical and physical, that are used to determine the composition of any substance," and what I am going to talk about this evening is the changes that are taking place in these techniques. I am going to talk about chemistry first, and then physics, though it is very doubtful where one ends and the other begins.

But before discussing details, I want to make two very important general points—chemical analysis is largely empirical in outlook and pragmatical in philosophy, so that theory usually lags behind practice; and it is a purely economic activity, nobody does it for fun or in the pursuit of pure knowledge. In Universities people study, for example, complex polyacids, because they are intrinsically interesting—to the people who study them. These may go on and show that some of the polyacids have properties that permit them to be used in analysis—that is analytical chemistry. But chemical analysis is practical and, concerned with economics, only studies polyacids in so far as they will make for quicker—and that means cheaper—or more accurate analyses. I expect I shall return to these points again, but whether I do so or not they are implicit in anything that I shall say. To an industrialist, chemical analysis is not—or ought not to be—an overhead, it is part of the cost of production and so is in the same category as plant maintenance. It is something you do to keep up and increase output while ensuring that the product is of the proper quality; even the activities of the public analysts and consultants are ultimately of this kind, and it is the duty of the head of the laboratory to see that his service is efficient and economical. In considering how he is to do this he arrives at two conclusions. (1) What he is producing is information, and every piece of information he supplies has a cash value; and somebody has to decide what his information is really worth. (2) That the most expensive ingredient in almost every analytical report is *laboratory time*. As he probably can do little about item (1) (it is the customer, not the supplier, who ultimately decides how much any product is worth), he

* Presented at the meeting of the North of England Section on Friday, October 30th, 1959.

wisely devotes himself to (2), and asks himself "How can I supply analytical information quicker than I do now, without loss of necessary accuracy?" There are actually two ways he can approach this problem; the first we are not concerned with tonight. It is to ask the question "Are all these analyses really useful?" and to arrive at the answer he will have recourse to statistical analysis in some form. The second approach is to look for new methods of analysis, and ultimately this is the driving force behind most of the research in analytical methods now going on. I know that some new methods are devised to be more accurate than old ones, and this is particularly so in organic analysis, but even then that accuracy has an economic value—and, moreover, most of the new organic methods are relatively rapid. But, generally, improved accuracy is not the chief point; either information is wanted on new subjects or information on old subjects is wanted quicker. How the information is being obtained will be the subject of the rest of this talk, and I propose to divide it into two parts, (1) largely chemical and (2) largely physical.

In the chemical field, I want to look at the progress being made in several lines. They are—

A. Micro analysis.

B. New reagents, including the non-specific chelating reagents.

C. Use of non-aqueous solvents.

D. Electrochemistry.

It is arbitrary to divide up the subject in this way; an actual analysis may spread over two or three categories, but for convenience we can use this classification.

Micro analysis of course is not new, but its use is increasing. It is not only as accurate —when applied to organic analysis—as macro analysis; it is quicker. It needs a manipulator with a very nice pair of hands, who need not be a very skilled chemist—in fact need not be a chemist at all. I remember during the second World War we had a girl who could complete 8 or even 10 micro Dumas nitrogen determinations per day, on quite awkward samples, but whose knowledge of chemistry was rudimentary. On the other hand, not everybody has the right temperament—apart from manual dexterity—to make a micro analyst. It is true that the endeavours of Belcher and others have much simplified the technique, and that there are automatic furnaces—at a price—that are very satisfactory, and much of that aura of sanctity that used to surround micro analysis is disappearing. Still, it is an exacting technique, and I am not sure whether a change to semi-micro analysis is much of an improvement. Advances are, however, always being made. Mettler-type balances make weighing simpler, for a start; the use of analysis by functional groups instead of for elements is increasing; the Schöniger method of combustion in oxygen enormously simplifies the determination of halogens and sulphur, and when we have combusted our sample and caught the products of combustion there are all kinds of clever ways we can use to complete the determination, and we ought to consider what justification we have for continuing to use some of the older methods. For example, every time I see somebody carrying out a gravimetric sulphate determination by barium sulphate, either on the micro or on the macro scale, I ask myself how much progress has really been made since Berzelius said that Swedish filter-papers (as "used by all the most clever chemists") were the best in the world.

We all know that the gravimetric method is tedious, we all ought to know that it is largely empirical, and only gives the right answer by compensation of errors. I grant that it is reproducible, but that is only half the story. Recently several quicker ways have been described. Archer[1] dilutes his aqueous solution with acetone, adds a little dithizone, which is soluble in acetone, and titrates with 0·01 *N* lead nitrate of which 1 ml is equivalent to 0·15 mg of sulphur in solution. As long as there is any sulphate, the lead is precipitated; as soon as there is a minute excess of lead, the green dithizone turns pink. Of course there have to be no heavy metals present, such as zinc, which can so easily be extracted in traces from some kinds of glassware, but it is very easy to clean up the solution by passing it through a column of base-exchange resin. This method can also be used for larger samples simply by diluting the solution and taking a small aliquot, as we need no longer be frightened by the bogy of introducing errors through successive dilutions. With proper use of calibrated apparatus, the errors introduced by dilution are insignificant compared with other errors in an analysis.

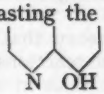
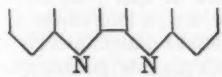
There are two other methods of titrating sulphate that we have found useful, one depending on lead and the other on barium as the reagent. Both are interesting and exemplify the fresh approaches being made to this kind of test. In the first,[2] an ingenious use is made of an oxidation - reduction system to locate the end-point. To the solution containing sulphate one adds a few drops of a very dilute solution containing ferro- and ferricyanide, then alcohol to lower the solubility of the lead sulphate, and titrates with dilute lead nitrate solution. As long as sulphate is present, the ratio of ferro- to ferri- remains constant and the redox potential of the solution measured by a platinum electrode is also constant. When all the sulphate is precipitated, the next drop of lead precipitates lead ferrocyanide, leaving ferricyanide unaffected, and there is a sharp change in the potential of the indicating electrode. This system works very well: we have recently been doing some phase-rule studies that necessitated dozens of determinations of sulphate ion, and all of them were made in this quick and accurate way. Of course the method is not free from interferences, phosphate interferes, calcium interferes, and both these ions can interfere in another useful titrimetric method for sulphate—titration with barium chloride solution with alizarin S as an adsorption indicator.[3] To sum up, there is not as yet a volumetric method for sulphate as free from interferences as the old gravimetric method, but there are several volumetric methods of fairly wide application, and we ought to ask ourselves "Should I be spending time on a gravimetric method for this particular sample, or should I be saving time by using a volumetric method?"

I have wandered rather a long way from micro analysis, but I wish to make the point that in both micro and macro analysis, gravimetric methods ought to become less and less common; this will not result in loss of accuracy and will achieve a vast saving of time. Only too often gravimetric analysis is a kind of sacred cow that wanders about in the laboratory helping itself to samples that it slowly consumes, and gives in return—what? A wholly misplaced trust in the idea that because we can weigh accurately, the analysis is accurate; but how often do we *really* know that the solid we have precipitated is pure and that precipitation is complete? No precipitate is quite insoluble, and almost every precipitate tends to drag down other substances with it; sometimes it is fairly easy to purify the precipitate, but not always. One can almost make a general statement that if possible one should avoid gravimetric determinations.

Nowadays there are so many ways of measuring quantities without using the balance that, particularly for small amounts, there is usually some better way of doing it. Volumetric analysis, either with indicators or electrical methods of indicating the end-point, spectrophotometry, polarography, should all be considered before resorting to the tedious business of precipitation, washing, re-dissolving, re-precipitating, washing again, drying, or calcining, cooling and weighing that constitutes gravimetric analysis.

I must pass on to a brief consideration of some of the new reagents. They illustrate rather well the empirical nature of chemical analysis. Anything goes, as long as it leads to the correct answer. Some day somebody will write the fascinating history of analytical reagents, and I believe that it will be shown that most of them were discovered in the first place by accident and that development work of a secondary kind was systematic. I do not think that Tschugaeff was trying to find a reagent for nickel when he discovered the intense crimson compound of nickel with dimethylglyoxime, but the man who first applied it as a highly selective reagent for palladium was surely guided by his knowledge of the periodic table. The first observation that 8-hydroxyquinoline could have analytical uses was certainly accidental. 8-Hydroxyquinoline potassium sulphate was originally used as a disinfectant of the intestinal tract, and in a note in *The Analyst* in 1918[4] it was pointed out that the solution was occasionally turbid and that the turbidity or precipitate was due to *copper*. It was suggested that the drug might have uses as a sensitive reagent for copper, and the next paper (in 1927) was also on copper; its use for aluminium and beryllium was 2 years later. Callan and Henderson, who first applied sodium diethyldithiocarbamate as a colorimetric reagent for copper, did not invent a new reagent, they exploited an observation that the chemical—already in production commercially—turned yellow if the least trace of copper got anywhere near it. Thioglycollates were not invented as colorimetric reagents for iron, and when Schwarzenbach began his classical investigations on ethylenediaminetetra-acetic acid, he did not have the analyst in mind. In 1933, Lundell of the Bureau of Standards in a fascinating paper[5] on the "Analysis of Things As They Are," a paper that ought to be prescribed reading for every analyst, said that in heaven there will be a shelf with 92 reagents

on it, number 13 being specific for aluminium, number 26 the sure shot for iron, number 39 the unfailing remedy for yttrium, and so on, but Lundell knew that this had not arrived yet. And we know now, thanks to the work of Irving and others, that it is not unlikely, it is impossible. True, we can make a selective reagent behave specifically in some cases by the skilful use of masking reagents, and a modern inorganic analyst must devote considerable attention to the equilibria that are involved. It is still in a sense true that any fool can do the determinations if an analyst tells him how to do the separations, but to-day our means of doing the separations are so much more varied than they used to be. I am not sure whether, when all the froth of papers on EDTA has subsided, it will not be found to be almost as useful a masking reagent as it is a titrant; for example, at the right pH, beryllium is the only metal precipitated as a hydroxide in presence of EDTA, and ammonium magnesium phosphate is almost the only phosphate. This leads us to the present-day idea that you separate only what you want to determine, *i.e.*, by precipitation or extraction, and leave all the other constituents behind. That is, separations tend to be more specific and more complete than they used to be.

But even though there is now some measure of system in the devising of organic reagents for metals, there is none in forecasting the occurrence of insolubility in inorganic compounds. We all know that the group  will combine with almost everything, that  is specific for copper and that if you put large groups too near the N steric hindrance will prevent compound formation from taking place, but so far as I am aware, we don't *really* know what confers low solubility in water on one compound and not on another. And from time to time fascinatingly improbable compounds turn up and solve a previously difficult problem. The triple uranyl acetates permitted sodium to be readily determined; $Cd[CS(NH_2)_2]_4Cr(CNS)_5OH.H_2O$ is almost insoluble, whereas the corresponding zinc salt is soluble. Who could have forecast that $[Co(NH_3)_6][(H_2O)_2Be_2(CO_3)_2(OH_3)]$ is readily prepared and is insoluble?[6] It is a compound of an unusual type and it may make a useful contribution to the analytical chemistry of beryllium.

But what we analytical chemists need more than lots of new and better reagents is more courage and skill in dropping some of the old ones. It is useless to devote time and labour to attempts to precipitate pure ammonium molybdophosphate from solutions containing lime, iron, vanadium and all the rest; it is an unrewarding exercise to argue about the best conditions for the titration of nickel with silver nitrate and cyanide; it is lost labour to go on endeavouring to determine small traces of copper in presence of cobalt with diethyldithiocarbamate years after Hoste has discovered that 2:2′-diquinolyl is specific for copper. We should be much more courageous in dropping hoary old methods and using new ones. How many British chemists use Lang and Furstenau's volumetric method for iron?[7]

A development that is as interesting as the new reagents is the increasing use of non-aqueous solvents, instead of water. These solvents are used in two ways, as a medium for volumetric titrations and for extracting various substances from aqueous solutions. Actually, neither is new. Alcohol has been used as a solvent for fats, and mixed with toluene as a solvent for many oils, before titrating the weak organic acids that they often contain, partly because these substances are not soluble in water and partly because it is easy to titrate weak acids in alcohol, with phenolphthalein as indicator, but to-day there is increasing use of non-aqueous solvents in volumetric analysis, for example, glacial acetic acid. We usually think of acetic acid as a weak acid soluble in water, but we can think of it as an amphiprotic solvent that can behave in two ways—

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$$
$$CH_3COOH + H^+ \rightleftharpoons CH_3COOH_2^+$$

It can either donate or accept a proton, just as water behaves in two ways—

$$HOH \rightleftharpoons H^+ + OH^-$$
$$H_2O + H^+ \rightleftharpoons H_3O^+$$

Now when a base dissolves, it can only show basic properties to the extent that protons are present for it to accept, and the more protons that are available, the more basic will

become the base. Acetic acid is far more protogenic than water, and hence the reaction $C_{10}H_7NH_2 + CH_3COOH \rightleftharpoons C_{10}H_7NH_3 + CH_3COO^-$ goes far further to the right in acetic acid than the similar reaction with water, so we can titrate our naphthylamine, as if it were a strong base, with perchloric acid in glacial acetic acid as titrant. In fact, in acetic acid all bases with $K\alpha > 10^{-12}$ are equally strong. This is called the *levelling effect*. But we may not want all our bases or all our acids to behave alike, we may want to differentiate between them. And indeed this is possible. By proper choice of aprotic, or mixtures of amphiprotic and aprotic solvents such as *iso*propanol and butyl methyl ketone, one can lengthen out the scale of acidity enormously, and potentiometrically titrate a whole string of acids or bases of varying degrees of strength, one after another.

The use of solvents as extractives is also in part an old story; everybody knows you can extract numbers of metals from water by a chloroform solution of 8-hydroxyquinoline; Smales and I during the second World War showed how by proper control of pH one could separate iron and uranium in this way. Everybody is familiar with the fact that ferric chloride can be removed from a strong hydrochloric solution with ether; auric chloride and some other noble metal halides, *e.g.*, iodoplatinous acid, can be extracted very well with ethyl acetate.

There is no completely general theory that accounts for every case, but many such extractions can be regarded as acid - base reactions, in which the salt is an oxonium compound more soluble in the non-aqueous phase than in water. I want now to call your attention to a recent development, in which the solvent is quite strongly basic, *e.g.*, a long-chain amine. For example, a toluene solution of trinonylamine will extract chromate quantitatively from an acid solution, and separate it from chromic salts, and also extracts the uranate, permanganate, ferricyanide and silicomolybdate anions.[8] A further development that may have wide applications is to form salts that are extracted into non-aqueous solvents by adding basic dye-stuffs to solutions of complex anions. The solvent extracts the dye-stuff salt, but not the excess of dye-stuff. The colour of the non-aqueous solution is a measure of the amount of anion present.[9]

But I must leave the new reagents and pass on to the physical methods, starting with electrochemistry. The oldest uses of electrochemistry were in electro-deposition and in potentiometric titration, and, although a modern analyst has a fine array of apparatus, when he is carrying out a potentiometric titration, it is in a well-established fashion. The recent development that is significant is that the titrator now records its own result, either in the form of a titration curve (volume vs. e.m.f.) or as the reading of a "burette follower," which records the volume of titrant used. Coupled to some form of automatic sampler, these apparatus can be installed on running plant, and we are a large step further towards automatic control. Actually, although automatic titrators are in use on plants, they have certain disadvantages, one of which is that they use large volumes of expensive standard solutions; the next step in this direction will possibly be an application of *coulometry*. This has been surprisingly neglected in Great Britain, but in the U.S.A. very considerable use is made[9] of it, and we should be putting more effort than we are into coulometry in this country.

There is simply not time to consider electrochemistry further; the various derivatives of polarography are making great contributions to some kinds of analysis, organic as well as inorganic, but I want to press on to the other purely physical methods, which are really revolutionising our ideas. The practice of chemical analysis has been profoundly modified during the last 30 years by a succession of improvements in detail, which we have been considering until now; what is now revolutionising our thinking in almost every branch of analysis is the possibility of chemical analysis entirely (or almost entirely) by physical means.

Many of us have been practising instrumental physical methods of analysis for years without being aware of it. Determining a refractive index in the course of the analysis of a fatty oil is just as much an instrumental physical method as determining the optical density of a hydrocarbon at a wavelength of 14 μ. The difference is that in the second example the apparatus is much more expensive.

There are two reasons for the increasing use of the more expensive "instruments" in an analytical laboratory (1) because you cannot obtain the desired results in any other way and (2) because you can obtain the results quicker; the combination of these two reasons has enormous persuasive power. The kind of instrument that I now want to describe usually—but not always—includes some stage of electronic amplification, and it is a most striking tendency of the present time that very often the final result is displayed as an electrical signal. This

is important outside the laboratory, as well as inside, because an electrical signal may perhaps be amplified and used to control a process, or, if not, may be displayed on a dial or chart so that the process operative can see it and act on it without having to wait for a sample to be taken to a laboratory, analysed and reported on. In the first case, the analysis is almost instantaneous and often continuous, in the second the delay must be appreciable; in a modern continuous process this can make the difference between success and failure. The increasing industrial use of infra-red measurements to control processes is a good example of successful application. This is but one instance of the rapidly expanding use of spectrophotometers in industry. In principle there is no difference between absorption analysis in the ultra-violet, the visible or the infra-red region of the spectrum; we think they are different because in the visible region we can perhaps regard a spectrophotometer as a legitimate descendent of a row of Nessler jars, and because the means of detection of radiation is different in the three cases. But what we do in each case is to measure radiation at a particular wavelength in order to ascertain how much passes through a particular solution; in fact, we measure transmission, but think in terms of the logarithm of the absorption.

Use of these instruments has had three very marked effects. First of all it has made readily accessible to us for measurement properties of the greatest analytical importance and so made possible many analyses that would formerly have been prohibitively complicated; *e.g.*, it is difficult to imagine the production of pure *p*-xylene as possible without the determination by infra-red absorption of the individual xylenes in hydrocarbons. Secondly, the use of monochromators has enabled many inorganic determinations to be made photometrically without recourse to chemical separations (for example, copper in presence of chromate or vice versa). Thirdly, it gives us a new and accurate method of determining a vast variety of organic and inorganic compounds for which slower methods were already known.

Now what accuracy can we obtain by this system? If it were low, we could use the method only for relatively small percentages, or for analyses of a low order of accuracy, and until quite recent years this was the view almost universally held. But it is not correct. Under favourable conditions one can measure radiation to an accuracy of about 2 per cent. of the amount present, that is, an error of not much more than 0·2 per cent. would be expected in a sample containing 10 per cent. of a component. But by measuring differentially, that is, measuring the difference between the sample and a known standard, vastly better accuracy can be attained. For example, phosphate can be determined by measuring differentially the colour given after converting the phosphate to $H_3PO_4VO_3.11MoO_3$, and on a fertiliser containing 12 per cent. of P_2O_5 the standard deviation is only 0·03; amazingly good for a routine measurement. In the analysis of aromatic hydrocarbons for *o*- or *p*-xylene, the error by infra-red analysis is only about 0·2 in 20 per cent.; 15 years ago an honest analyst reported an infra-red analysis as 20 ± 5 per cent.

Besides being quick and accurate, spectrophotometry in some cases gives us both sensitivity and specificity. It also readily lends itself to being combined with other techniques, such as chromatography. For example, in the determination of polynuclear hydrocarbons in the atmosphere, careful chromatographic fractionation is followed by final spectrophotometry in the near-ultra-violet region, preferably with a recording instrument.

I must at this point diverge and say something about chromatography—one of the brightest jewels in the modern analyst's crown. Invented—I think that is the word—many years ago, vastly modified by Martin and James. Dozens of analysts, employed in analysing hydrocarbons, esters, alcohols, phenols and essential oils, now wonder how they ever used to do their work before they had this technique. There is no time to say much about the older forms of chromatography, or the fascinating uses of ion exchange in inorganic analysis, but I must mention that revolutionary development—gas-phase chromatography. This is not the occasion to give any detailed description. The travelling gas behaves like the flowing liquid in ordinary partition chromatography and mixtures of volatile substances are separated in a manner analogous to the separation of carotenes and chlorophyll in the analysis of leaf pigments. In a few years devices of quite fantastic sensitivity have been made to determine the separated components as they flow from the column, so that on a sample of a cubic centimetre or so of gas—say mixed hydrocarbons—one can detect and determine as little as a few parts per million of some particular component. Development in the art of selecting suitable stationary phases has progressed so far that there are now few mixtures of volatile liquids—say with boiling points up to 300° C—that cannot be wholly or partly resolved and

hence analysed by this means, often in the space of a few minutes. Alcohols, esters, fatty acids, halogenated hydrocarbons, all are readily and quickly analysed by this method.

It is commonplace to analyse the C_2 to C_4 fraction from a petroleum cracker, containing as many as 11 or 12 hydrocarbons, in 20 minutes or less, and the sum of the results for each component will be between 98 and 102 per cent. This is more accurate than mass spectrography, quicker, simpler and cheaper. In fact, I venture to say there would be few mass spectrographs in oil refineries if vapour-phase chromatography had appeared 10 years earlier than it did.

A similar remark could be applied to X-ray fluorescence analysis as a possible replacement for emission spectroscopy in the inorganic field. But the story of the application of emission spectrography to analysis is extraordinarily interesting and illustrates so many points that it is worth while to spend a few minutes over it. Not the least interesting is that, although the first application of the method to chemical analysis was in 1826, it was not applied to the regular analysis of any product for another 100 years.

In many recent applications of physical techniques, the development of usable apparatus has rapidly followed the first academic indications that a property could be measured, but in spectrography many years elapsed before readily usable apparatus was made, and even then it was a long time before the method came into regular use.

The first real spectroscope was made by Fraunhofer, who in 1817 combined a slit, a crown-glass prism and a theodolite. In 1826, Fox Talbot, the inventor of photography, wrote a paper on "Some Experiments on Coloured Flames," in which he examined with a prism and a slit the *red fire* used in theatres. It gave a beautiful spectrum with many bright lines. Talbot wrote "The red ray may be the effect of strontia, since Mr. Herschel in 1823 found in the flame of muriate of strontia a ray of that colour. If this opinion should be correct and applicable to the other definite rays, a glance at the prismatic spectrum of a flame may show it to contain substances which it would otherwise necessitate a laborious chemical analysis to detect." Eight years later, in 1834, he wrote "It is difficult to distinguish the lithia red from the strontia red by the unassisted eye. But the prism displays between them the most marked distinction that can be imagined. The strontia flame exhibits a great number of red rays, not to mention an orange and a very definite bright blue ray. The lithia exhibits one red ray. Hence I hesitate not to say that optical analysis can distinguish the minutest portions of these substances with as much certainty, if not more, than any other known method." This is the first definite statement on the possibility of spectrochemical analysis. In 1860 there was Bunsen's paper on "Chemical Analysis by means of Spectral Observations," and in 1861 Bunsen and Kirchhoff by means of the spectroscope discovered rubidium, followed shortly by caesium. Various other elements were first discovered by spectrographic means, and in 1874 Lecoq de Boisbaudran[10] published an atlas of spectra and wrote as the opening words of the text "*Le spectroscope est maintenant l'auxiliare indispensable de tous les chimistes.*" This was manifestly not true, as most chemists assiduously neglected this aid. Perhaps the method was too sensitive; until electricity was readily available the technique was difficult; until photography was applied later in the century, the important ultra-violet spectrum was inaccessible, and the complex spectra of transition elements could not be handled. But in 1904, Hilger's had evolved the constant-deviation spectrograph, in 1909 the first convenient photographic medium-size spectrograph appeared, and then in 1912 the large-quartz spectrograph in very much the form that we know it to-day.

It may be said that with this last instrument the modern spectrograph arrived and really rendered quantitative spectrographic analysis possible. I have included the above abridged history because it is interesting as illustrating the number of practical steps between the first idea and the realisation. Not less interesting—and this is more difficult to understand—is the apparent reluctance with which all except a very small handful of enthusiasts —Ramage, Hartley, de Gramont—greeted the "new" method. In 1923 de Gramont was almost alone in using spectrographic methods for quantitative analysis—and apparently his apparatus was not provided for him by his university, but bought out of his own pocket. There are probably three reasons for this slow appreciation of the possibilities of this method. Very few analysts had the academic background to realise what it was that they were being offered, because this was the first such technique to appear, and the underlying ideas were too foreign to their life. Secondly, there were already more or less adequate ways of analysing most commercial materials; after all, we made and analysed copper, alloys and steels long

before spectrographs became available. Thirdly, the instruments at first sight appeared to be expensive, and—as some of us can remember too well—laboratory assistants were cheap.

In another 10 years, however, the picture was different. To quote the A.S.T.M. bibliography on spectrochemical literature "the contagion quickly spread through the civilised world and papers appeared in many languages," and the cause was the rapidly increasing scale of metallurgical operations, which necessitated more and quicker tests to control products sold to more and more exacting specifications. By 1923 one brass foundry in the U.S.A. was using a spectrograph qualitatively, and shortly afterwards routine checks on the purity of zinc and cadmium were being carried out in Norway and Belgium. In England, Main-Smith wrote an important paper in 1934 on applications of spectrography in the non-ferrous metal industry, but its use for quantitative control of both light alloys and copper had been well established at I.C.I. Metals by 1931. In 1930 Twyman and Fitch read a paper—the first in English since 1875—on ferrous metal analysis. They described the use of a logarithmic sector, so that the *length* of the line is related to the amount present, and plotted the lengths of, *e.g.*, a nickel line against the amount of nickel in a series of standard samples. In 1933 a second paper by Twyman appeared, dealing with the determination of Ni, Cr and Mn by the internal-standard method. This important paper was read at a meeting of the Iron and Steel Institute at Sheffield, and it would be a salutary exercise for all analysts to read the discussion. Perhaps only the authors realised that they had announced the end of an era —the era in which only chemistry was of consequence in industrial inorganic analysis. Twenty-six years afterwards one wonders how much longer chemistry will be regarded as the dominant partner.

It was a great step forward when it was firmly established that the ratio of the intensity of a suitable iron line and of a suitable line of the alloying element were logarithmically related. These intensities are estimated by measuring the darkness of the lines on the photographic plate, and even after good microphotometers were produced about 1939 or 1940, accuracy was not very high, partly because of the nature of the photographic plate, and considerable ingenuity has been shown in devising expressions that would, so to speak, straighten up the curved graphs obtained and make them into straight lines. After 1939 excellent optical instruments were produced with which standard spectra and the spectra from samples could be projected and compared side by side and selected lines in the spectra measured in a microphotometer. If necessary, this microphotometer can be coupled to a recorder and the optical densities of a whole series of lines recorded one after another automatically. That is probably as far as we are likely to go for some time in photographic analysis of emission spectra. The accuracy attainable is still not very high, perhaps, at best, 5 per cent. of the amount present. This is adequate for smaller amounts of alloy elements and what are called *residual elements*, but is rather inadequate for the accurate determination of major alloy elements.

In the United States this process was not regarded as quick enough and a vast amount of work was put into the manufacture of spectrophotometers that would simultaneously determine a number of elements. This is done by mounting a series of slits round the exit circle of a very large grating spectrometer. Each slit is mounted at the point where a particular line emerges and behind each slit is a photomultiplier tube. Thus, some suitable line of the iron spectrum is chosen as the master reference line and a series of lines, one for each element required. The instrument electronically compares the intensities of the various lines with the iron line and can present the results either as lines on a bar chart, which give the ratios of the elements to iron, or it can even type out the percentages of elements present so that the entire analysis of the specimen can be presented in a few minutes once the instrument has been bought, installed in a thermostatted room and calibrated. Numbers of these expensive and complicated instruments have been made in England and are in use in the ferrous and non-ferrous metal industries and in the uranium industry. Of course, this instrumentation does not really get over the inherent difficulties of the emission spectrographic method. It is still one of the less accurate methods of analysis and it is rather curious to note that progress in emission spectrography is now most conspicuous in a form of spectral analysis that thirty years ago seemed moribund—flame photometry. In suitable instruments —which need not be too complicated—it is surprising what accuracy can be achieved; for example, by using the method known as "close bracketing" (a kind of differential method) one can determine potash in fertilisers, up to 20 per cent., as accurately as by platinic chloride. For some unexplained reason accuracy falls off above the 20 per cent. level.

Besides increasing use of sensitive instruments containing monochromators, great progress is being made in *absorption flame photometry*, which is almost specific for the element in question and is more accurate than emission flame photometry. The latest development is use of a *very* hot flame of cyanogen burning in oxygen; not the sort of thing I feel I should like to have in my own laboratory, but it may develop further.

But I want to pass on to the most important development in inorganic analysis for many years—X-ray methods, and particularly the use of X-ray fluorescence.

The earlier methods of X-ray analysis followed the general line of bombarding the sample with a concentrated stream of electrons so that it emits the X-rays characteristic of the elements contained. These were diffracted at a crystal surface and recorded. The method was difficult; the sample had to be pasted on to the target of an X-ray tube, which then had to be pumped down, and it never attained any great popularity, though undoubtedly it was of great use from time to time. At the same time, say from 1935 to date, the use of X-ray diffraction crystal analysis steadily increased, and it is not difficult to see why it should. To take the simplest possible instance—if an analyst is asked to analyse a mixture of sodium sulphate and potassium chloride, he can by chemistry determine the sodium, the sulphate, the chloride and the potassium. He can then go on to do some calculations that lead him to the conclusion that what he has is sodium chloride and potassium sulphate, or sodium sulphate and potassium chloride, but these calculations break down if several other salts that contain the same components are present or if the salts are present in molar ratio, and it may well be that the real interest in the sample is not in how much chloride, how much potassium and how much sulphate it contains, but in the simple question "Is this potassium chloride and sodium sulphate or is it potassium sulphate and sodium chloride?" The analyst, if he were ingenious and possessed a polarising microscope which he knew how to use *might* be able to answer this question. The X-ray crystallographer with a diffraction camera could answer it categorically and fairly quickly. This, of course, is an absurdly simple example; the real value of the technique is usually in far more complicated cases. For example a firebrick consists of 90 per cent. of silica and has been heated. Above a certain temperature the silica will have been converted to tridymite, which can be identified readily by X-ray diffraction, but probably by no other means, and it may be very important to know to what temperature the firebrick has been heated. A pigment may be largely titanium oxide. The chemical analysts tell us that it contains 85 per cent. of titanium oxide. What we really want to know may be, not how much titanium oxide is present, but how much is rutile and how much is anatase, and again X-ray diffraction can tell us the answer.

In the Billingham analytical laboratories, the method is being continually used for the qualitative or semi-quantitative analysis of boiler deposits, deposits in chimneys and flues, mineral specimens and so forth. A practiced man learns to recognise at sight numerous X-ray patterns and if he cannot recognise them there is a fairly standard system of reference so that they can be identified readily from tables. After all, why should we do qualitative analysis and destroy the sample in the process when the X-ray crystallographer can tell us nearly as much in about one quarter of the time and hand us our sample back when he is finished with it? There is, of course, a catch in it, a catch of rather serious nature; in fact, there are two catches. The first is that colloidal materials give no indication to the X-ray crystallographer at all of their nature and the second is that there may be ambiguity if too many related crystal forms are present. There was, some years ago, a dreadful case when after examination by X-ray diffraction a flue dust was said to consist of zinc oxide. This so surprised everybody that we were asked to resort to good old-fashioned wet analysis to find out what the stuff really was. It was mostly amorphous carbon, soot in fact, and it contained about 1½ per cent. of zinc oxide, but that being the only crystalline substance present was the only one the X-ray spectrographer could find. This is a simple example of two facts, the first of which cannot be over emphasised, that there are no infallible methods of analysis, the second—dare I mention it?—is that from time to time even quite competent analysts will commit the most frightful of blunders.

So far we have been considering X-ray diffraction and emission, but since about the end of the last War apparatus has become available that makes analysis by fluorescent X-rays very simple indeed.[12] If X-rays of very short wavelength are generated in a tube with a tungsten or molybdenum target and these X-rays are used to irradiate a sample, the elements of the sample will themselves be excited until they emit so-called secondary or fluorescent X-rays. They are called fluorescent X-rays because the phenomenon is almost exactly

analagous to fluorescence as ordinarily understood. When irradiation of a molecule by light of fairly short wavelength causes the molecule to emit light of a longer wavelength, the wavelength of the emitted light is characteristic of the compound in question and, in the same way, the wavelength of the secondary or fluorescent X-ray is characteristic of the element in question, because here we are dealing not with a molecular property but with an atomic property; thus it does not matter under what sort of combination the atom finds itself it always emits rays of the same wavelengths. The number of possible wavelengths for each element is quite small. These rays can be collimated and diffracted at the surface of a crystal mounted in a goniometer. The angle of diffraction of the beam of X-rays will depend on their wavelength and their wavelength depends on the atoms present, so that measuring the angle of diffraction will tell us what elements are present and the amount of X-rays at the particular angle will tell us how much of each element is present. We can thus make a qualitative and quantitative analysis of our sample by means of fluorescent X-rays. It is also interesting to know that here we have another physical method of analysis that is independent of the weight of the sample, because as long as the same area of sample is exposed to the beam of primary X-rays and the sample is so thick that no X-rays pass right through it, the same amount of secondary X-rays will always be produced. The sample may be powdered or it may be in solution or again it may be a homogeneous solid, such as a glass or cast metal; it makes very little difference. Once the calibration has been made it is possible to do an analysis of, for example, a catalyst for its heavy-metal content in no more than 3 or 4 minutes. The procedure is to measure the intensity of the X-rays diffracted at the angle peculiar to the element in question by means of a Geiger counter or scintillation counter and determine the amount of X-radiation emitted in a given time. Another interesting point about this system of analysis is that it can be made independent of chemically analysed samples—in some cases. If the sample is a solution or a suitable powder one can add a known amount of the element in question and again determine the number of counts at the proper angle. A little arithmetic then tells us from the ratio of the two counts how much of the element was originally present. But in general it is usual to calibrate the instrument with analysed samples; indeed, once again, somewhere behind the magnificent array of electronics, scintillation counters, dials, power packs, recorders and all the rest of it, somewhere in the background there is a chap, usually (I regret to say) nowadays in his middle-age, who knows his periodic table and knows the classical methods of analysis and knows how to use his hands, and as far as I can see it is still going to be some time before we can get away from him. Unfortunately, *he* is usually middle-aged, and it is apparently one of the most difficult things in the world to train his successors, and here is one of the analytical administrator's perpetual problems.

A fairly eminent American chief analyst said to me some time ago, "20 years ago if I were given a can of paint on a Monday morning, by Wednesday morning my boss expected to know the mineral constituents of the pigment, what oil had been used, and the nature of the thinners. Nowadays if I give a man a can of paint on Monday, by Wednesday morning he has not even decided what wavelength to use." This is really unfair because the can of paint at the present day is a vastly more complicated business than the old linseed oil - turpentine - mastic mixture that my acquaintance had in mind, but as a man who sometimes wants the analysis of an out of the way sample in a hurry I do indeed sympathise with his rather bad-tempered remark, and no more than he do I know how to convert a present-day graduate into a good old-fashioned "wet" analyst. (In America a "wet" analyst is one versed in the classical tradition in which considerable use is made of reactions in aqueous solution.)

I am inclined to think that in large laboratories the solution of the problem may lie in extending the importance of the standardisation section, which will have to supply a stream of accurately analysed samples to be used in standardising the apparatus that will be used for doing most of the analysis.

I must now draw to a close, conscious of the fact that I have not mentioned several important topics, such as the methods that have only become possible since atomic energy began to be available, mass spectrography, nuclear-magnetic resonance and biochemical methods. These last have up to now been used only in the pharmaceutical industry and to a limited extent in the determination of certain vitamins. But if, as seems likely, the practice of adding potent bacteriostats and other biologically active substances to feeding-stuffs continues as part of the ever-intensifying effort to increase the productivity of agriculture, we may see their use increasing in fields where a few years ago they were almost unknown.

Nor have I said anything about another important influence on progress in chemical analysis—that is the increase in so-called "standard" methods of analysis, and here before I conclude I must make one or two observations. When we are dealing with arbitrary or empirical methods of examination, in which results have meaning only if they are obtained under given conditions, clearly the more closely defined the conditions the better. But this need not be true when we are determining a chemical entity. If the sample is to be analysed for arsenic, or copper, or uranium, or sulphur, the extent to which we need "standard" methods is a measure of the extent of our ignorance. I do not deny that commercial considerations make it convenient and profitable that agreed methods should be available, if only because two parties are then more likely to get the same answer and so save an expensive dispute. Lundell[5] divided the analytical population into "determinators and analysts. The determinators, who are by far the more numerous, are of two kinds. First the common determinators who follow a method exactly without knowledge or concern as to the reactions involved and second the educated determinators who can handle systems containing one or perhaps two variables . . .," and it is for these people that we spend so much time preparing "standard" methods. To the accomplished analyst determining an element in an inorganic sample, such a method ought to be unnecessary and in fact is sometimes simply frustrating. The better the analyst in charge, the better able he is to write his own methods, which will commonly be found more satisfactory for the materials he is dealing with; if the right man is in the right place, he will often find himself saying "Yes, I know it says you dissolve 0·5 g of the sample in 10 ml of nitric acid, boil for 15 minutes, and all the rest of it; *we* are going to dissolve 0·25 g in 10 ml of hydrobromic acid, add 10 g of potassium bromide, dilute to a litre and measure the optical density at 375 mμ." I will further point out—and this is a thing far too little realised—that when you have standardised anything you have done your best to stop it developing; whether you realise it or not, what you are saying is "There has been plenty of development here, let us standardise what we have now." I have done my share in standardising methods both inside our own organisation and outside it, but it is work I always regard as expedient rather than commendable, and I have often thought that time would be better spent in studying the reactions rather than in "standardising" them.

In this context I will tell you a favourite anecdote of mine about an old Dutch friend who was visiting our laboratories some years ago. We were walking along discussing analysis in general and he suddenly stopped and said "Mistaire Vilson, I vill tell you an analyst." His English was fluent but occasionally not quite grammatical. So I said "I don't quite understand, but you tell me an analyst." And his reply was absolutely to the point, "An analyst is ven zay say zere is a standard method for so and so, and he vill say, 'Ja, but I know a better von.'"

In a recent editorial in *Chimie Analytique*,[12] Professor Charlot has summarised the analytical situation as he sees it in France. Perhaps two of his aphorisms are not entirely wide of the mark in this country—

S'il s'agit de problèmes classiques, le laboratoire est en general organisé pour effectuer les opérations correspondantes. Mais aujourd'hui les problèmes nouveaux sont prépondérantes.

De nombreux analystes devraient repasser par l'École.

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The Detection and Determination of Diphenyl and *o*-Phenylphenol in Concentrated Orange Juice by Gas Chromatography

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Chemical methods at present used for determining diphenyl and *o*-phenylphenol were found to be too slow and insensitive for routine tests on concentrated orange juice. Both compounds can be simultaneously determined in a 1-ml sample of juice by gas chromatography,. The experimental error, at a probability of 95 per cent., is $\pm 0{\cdot}4$ p.p.m. for diphenyl and $+0{\cdot}4$ to $-0{\cdot}8$ p.p.m. for *o*-phenylphenol, both compounds being present in concentrations between 1 and 10 p.p.m. Both compounds can be determined in about 35 minutes.

Since the amendments to the Public Health (Preservatives etc. in Food) Regulations in 1958, the use of diphenyl and *o*-phenylphenol as mould inhibitors has been permitted for citrus fruits, apples, pears, pineapples, peaches and melons; details of the permitted amounts are set out in Statutory Instruments.[1,2]

It is unlikely that these mould inhibitors would be deliberately added to concentrated orange juice, but the possibility of accidental contamination makes desirable a rapid and sensitive method for their determination.

The currently available analytical methods for diphenyl and *o*-phenylphenol were found to be unsuitable for routine use with concentrated orange juice. Tomkins and Isherwood[3] found it necessary to distil 2 litres of juice and used a complicated extraction technique for the determination. Cox[4] used essentially the same technique, but decreased the amounts somewhat and measured the ultra-violet absorption. Infra-red analysis was used by Boorman, Daubney and Martin,[5] but this method is time-consuming. Bruce and Howard[6] reduced nitrated diphenyl extracts, diazotised, coupled the product with 1-naphthylethylenediamine and then measured the optical density of the coloured solution so formed; this method took about 3 hours to complete. Kirchner, Miller and Rice[7] used a modified paper-chromatographic technique, but again the method took about 3 hours to complete and required large amounts of juice.

Chang, Horsfeld and Sandstrom's method[8] is qualitative, and other methods[9,10,11] were equally inconvenient for routine use with concentrated orange juice. Any procedure beginning with solvent extraction must be modified, because of the ease with which concentrated orange juice forms emulsions; even when a juice has been diluted ten times, an emulsion frequently forms when it is shaken with organic solvents.

The simplest way of overcoming this difficulty was found to be by steam-distilling the diluted sample. Since the volume of distillate required for gas-chromatographic analysis is about 1 ml, the time taken to prepare the sample is short, and steam-distillation can be routinely used for the preliminary separation of diphenyl and *o*-phenylphenol from the orange-juice residue.

Samples of concentrated juice from various sources were examined to discover whether or not they contained naturally occurring substances that might interfere with the method, *i.e.*, substances having retention volumes so close to those of the compounds being determined that the areas under the required peaks could not be measured. Few of the juices examined contained interfering substances to any significant extent, and it was found that such compounds could be removed by a modified extraction technique, which was subsequently used whenever they were present.

Experimental

The gas-chromatographic detection of diphenyl was first attempted with use of a katharometer detector, but this was not sufficiently sensitive; chromatograms obtained from a Gas Chromatography Ltd. apparatus (mark IE 110, fitted with an argon-ionisation detector)

were finally used. The column was 6 feet in length and was packed with 20 per cent. w/w of Embaphase silicone oil on 100- to 120-mesh Celite. The temperature of the column was maintained at 160° C and the flow rate used was about 20 ml per minute. Lower temperatures and smaller amounts of stationary phase were tried, but the conditions stated above were found to give the best results.

For quantitative determinations by gas chromatography either the amount of sample placed on the column must be known or an internal standard must be used. With an argon detector, however, the amount of sample required is so small that it cannot at present be precisely measured; the measurement of small volumes, even of mobile liquids, taxes the resources of most experimenters. The extracts of orange juice used for these determinations, being viscous oils, presented even greater difficulties of measurement, and an internal standard was consequently used.

The choice of an internal standard was not simple. Ideally, the standard should have a retention volume close to those of the compounds being determined, but not so close that the peaks interfere; it should behave chemically and physically in a manner similar to those of the compounds being determined, and its peak should preferably be on a part of the chromatogram in which peaks produced by naturally occurring compounds are unlikely to appear. In the determination of diphenyl and *o*-phenylphenol, thymol was found to be a suitable internal standard. Naturally occurring compounds sometimes interfered with the peak for thymol, but the area under the peak could still be found by recording a chromatogram from an extract containing no added thymol and then comparing it with a chromatogram recorded after thymol had been added. Thymol boils in the same range of temperatures (233° to 275° C) as do diphenyl and *o*-phenylphenol; the vapour pressures of these substances at any other temperature are therefore similar, so that all three evaporate at about the same rate. Errors caused by evaporation losses during handling are therefore minimised.

Method

Reagents—

Internal-standard solution—Dilute 25 ml of a methanolic solution of thymol (0·008 g per litre) with 75 ml of water. This solution contains 2 μg of thymol per ml.

Chloroform.

Hydrochloric acid, dilute.

Treatment of sample—

A 1-ml portion of concentrated orange juice was diluted with 4 ml of water, 1 ml of internal-standard solution was added, and the mixture was steam-distilled in a Quickfit & Quartz semi-micro apparatus (assembly No. 19 MU). A 1-ml portion of distillate was collected and then shaken with 0·5 ml of chloroform in a 10-ml separating funnel. To break the emulsion that sometimes formed, the mixture was acidified with dilute hydrochloric acid and was then allowed to separate. The chloroform layer was run on to a watch-glass. (At this stage, it is important to ensure that the chloroform extract contains no water. If present, water can be seen as small globules and can be removed by slowly pouring the chloroform solution on to another watch-glass; the water adheres to the first watch-glass.)

Injection of sample—

If the chloroform solution is allowed to evaporate, the residue is a viscous oil that cannot be easily manipulated; alternatively, if the chloroform solution is placed on the column the amount of chloroform present saturates the column and the peak produced swamps the region of interest on the chromatogram. In order to overcome the difficulty in injecting samples, the technique described below was devised.

About 10 mg of the column packing (Celite *plus* liquid phase) were mixed with the chloroform solution on the watch-glass, and the chloroform was allowed to evaporate, leaving the residue absorbed on the powder. The powder was scraped from the watch-glass on to a piece of clean paper and was then poured on to the column as soon as all the chloroform had evaporated. (The powder has a slight tendency to adhere to the watch-glass, but, once it has been removed therefrom, recovers its free-flowing property.) The gas-inlet tube was re-connected as rapidly as possible after the sample had been placed on the column.

The transfer of powder from watch-glass to column can be accomplished in this way with little loss of material, but the gas-inlet tube must be replaced in the shortest possible time; inaccuracies will otherwise occur, owing to loss of sample by evaporation and to spread of sample along the column by diffusion before the gas supply is re-connected.

When this procedure is used, almost the same weight of sample can be analysed on successive occasions and the chromatograms have peak heights varying by not more than 1 cm from one analysis to the next. At the end of a run, the added Celite can easily be removed by loosening the gas-inlet tube and momentarily turning on the gas. This blows the Celite away from the glass-wool plug on the top of the column, which is then again ready for use.

Results

Table I shows a comparison between the amounts of diphenyl and *o*-phenylphenol added and those found from the chromatograms.

Table I

Recovery of added diphenyl and *o*-phenylphenol by the proposed method

Diphenyl added, p.p.m.	Diphenyl found, p.p.m.	*o*-Phenylphenol added, p.p.m.	*o*-Phenylphenol found, p.p.m.
0	0	2	1·6
	0	5	4·75
1	0·93	0	0
	1·13	1	1·15
	1·33	0	0
	2·04	2	1·75
2	1·7	0	0
	1·65	2	1·9
	1·62	2	1·56
	2·1	0	0
	1·93	0	0
4	4·2	4	3·8
	4·2	4	3·6
5	5·15	2	2·26
	4·8	5	4·6
6	6·25	6	6·08
	6·0	0	0

The standard deviations of the differences between the amounts added and found were 0·21 p.p.m. for diphenyl and 0·31 p.p.m. for *o*-phenylphenol, so that the experimental error (at a probability of 95 per cent.) can be taken as being about $\pm 0{\cdot}42$ p.p.m. for diphenyl and $\pm 0{\cdot}62$ p.p.m. for *o*-phenylphenol. The observed readings for the latter substance were, on average, 0·2 p.p.m. low, and an estimate of the adjusted experimental error can be taken as being $+0{\cdot}4$ to $-0{\cdot}8$ p.p.m. The low average in the determination of *o*-phenylphenol may be caused by the great difference between the retention volumes of thymol and *o*-phenylphenol.

Fig. 1 shows the chromatograms obtained from a sample of Israeli orange juice before and after 2 p.p.m. of thymol and 4 p.p.m. each of diphenyl and *o*-phenylphenol had been added. Both chromatograms were obtained from 2-ml portions of concentrated juice, and the sensitivity of the instrument was adjusted to give easily measurable peaks.

Fig. 2 shows the chromatograms obtained from 1-ml portions of a Spanish orange juice before and after 2 p.p.m. each of thymol, diphenyl and *o*-phenylphenol had been added, the conditions finally chosen being used. The instrument was set at maximum sensitivity in order to deal with the smaller amount of extract. *o*-Phenylphenol could not be determined directly in this particular juice because a compound having practically the same retention volume was present. The problem arising with such a chromatogram is whether or not the peak is produced by *o*-phenylphenol.

In this instance, two lines of approach were used and both proved that the compound producing the large peak on curve A in Fig. 2 was not *o*-phenylphenol. The sample was examined by a solvent-extraction method based on the chemical properties of phenols. The steam-distillate was extracted with 1 ml of hexane, and the hexane solution was then shaken with 1 ml of dilute sodium hydroxide solution. After separation, the alkaline layer was acidified, any organic components were extracted into chloroform, and the chloroform was evaporated with Celite. The chromatogram obtained after this treatment (see Fig. 3,

curve A) showed that the interfering peak had been removed. The fact that *o*-phenylphenol and thymol were unaffected by the extraction is demonstrated by curve B in Fig. 3; this chromatogram was obtained after 2 p.p.m. each of thymol, diphenyl and *o*-phenylphenol had been added to the concentrated juice.

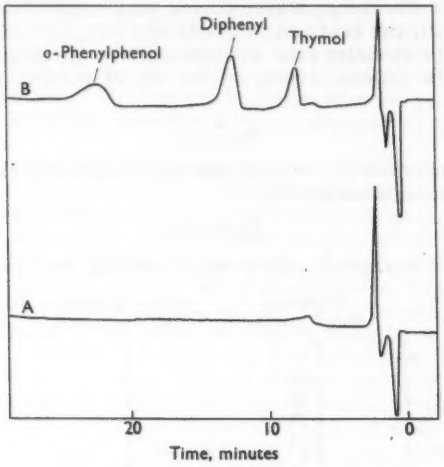

Fig. 1. Chromatograms obtained from Israeli orange juice: curve A, 2 ml of concentrated juice; curve B, 2 ml of concentrated juice *plus* 2 p.p.m. of thymol and 4 p.p.m. each of diphenyl and *o*-phenylphenol

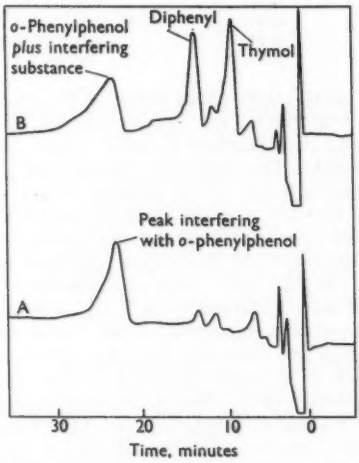

Fig. 2. Chromatograms obtained from Spanish orange juice: curve A, 1 ml of concentrated juice; curve B, 1 ml of concentrated juice *plus* 2 p.p.m. each of thymol, diphenyl and *o*-phenylphenol

The problem was also examined spectroscopically, the steam-distillate being extracted with hexane, dilute sodium hydroxide solution and again with hexane. When the extract from juice containing added *o*-phenylphenol was examined with a Unicam SP500 spectrophotometer, two distinct peaks were found, one at 245 μ and another at 283 μ; this absorption

curve was identical to that for pure *o*-phenylphenol in hexane. The absorption curve for the similarly treated extract from the Spanish orange juice containing no added *o*-phenylphenol did not show these peaks, but showed a slight peak at about 260 μ.

The chloroform solution giving rise to the chromatogram shown in Fig. 2, curve A, was examined spectroscopically, but no major peaks were observed. This suggests that the peak interefering with the determination of *o*-phenylphenol may be a mixture of compounds.

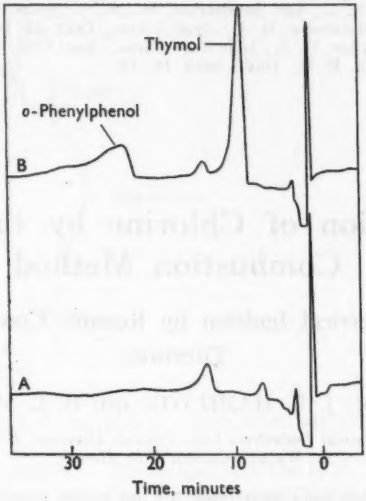

Fig. 3. Chromatograms obtained from Spanish orange juice after solvent extraction of the steam-distillate: curve A, 1 ml of concentrated juice; curve B, 1 ml of concentrated juice *plus* 2 p.p.m. each of thymol, diphenyl and *o*-phenylphenol

Discussion of the method

Diphenyl and *o*-phenylphenol were quantitatively determined from the chromatograms obtained by the usual method of drawing tangents to the peaks and calculating the areas of the triangles so formed.

When a number of the typical results shown in Table I were statistically examined, it appeared that the measured area under the peak for diphenyl was, within experimental error, proportional to the amount of diphenyl added to the concentrated juice. The difficulty in drawing accurate tangents to the peaks for *o*-phenylphenol increased the experimental error, but it also seemed that, on average, the result from a number of determinations was low.

On one occasion, a natural component that interfered with the peak for thymol was observed. However, since all batches were first examined without the addition of the internal standard, the position of and area under the peak for thymol added for quantitative determination could easily be found.

No compound that interferes seriously with the determination of diphenyl has been found in the concentrated orange juices so far examined.

It has been shown that diphenyl and *o*-phenylphenol present in concentrated orange juice can be determined with reasonable accuracy by using thymol as an internal standard. Only 1 ml of concentrated juice is required for determinations down to 1 p.p.m., and tedious extraction techniques are avoided.

I thank Dr. D. McAnally for his help with the spectroscopic analyses and Mr. J. M. Harries for the statistical analysis.

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The Determination of Chlorine by the Oxygen Flask Combustion Method

A Single Unit for Electrical Ignition by Remote Control and Potentiometric Titration

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A single unit has been developed for use in the oxygen flask combustion method; it permits samples to be fired electrically by remote control and, further, allows titration of the combustion products directly in the combustion flask. Examples are given of the application of the unit in determining chlorine in polymers and allied compounds, and it is suggested that the apparatus may have applications in other fields.

RECENTLY, much work has been carried out in this laboratory on the determination of chlorine and bromine in organic compounds by the oxygen flask combustion method. The work on chlorine has been described in detail elsewhere,[1] the method being, briefly, as described below. The test sample is wrapped in filter-paper and placed in a platinum basket; a filter-paper wick from the sample is ignited externally, and the combustion in oxygen is carried out in a closed separating funnel. This method has worked satisfactorily, but it seemed desirable, after experience of the test, to design a single unit in which (*i*) the sample could be electrically fired by remote control; this would obviate the potential hazards present with manual firing and permit the combustion to be initiated in a totally enclosed system, (*ii*) the firing adapter could be used continuously without replacement of firing element or fuse wire and (*iii*) the ionised halide could be absorbed and titrated extremely accurately in the combustion vessel by means of a full-scale automatic titrimeter; this would avoid possible errors incurred during transfer of the absorption solution before titration.

An apparatus incorporating these features has been designed, and full details of the method of construction and working procedure are given below.

METHOD

APPARATUS—

Combustion flask and firing adapter—The complete combustion flask and firing adapter together with the firing-spark source are shown in Fig. 1, and a more detailed diagram of the firing adapter together with the necessary constructional details are shown in Fig. 2.

The H.F. Tester (model T.1) for producing the spark used to initiate the combustion is manufactured by Edwards High Vacuum Company Limited, Crawley, Sussex; it is mains

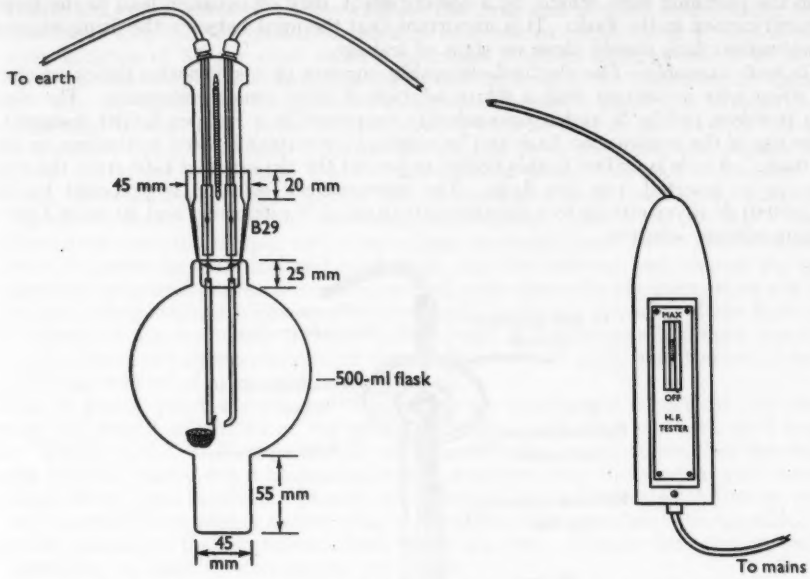

Fig. 1. Combustion unit and spark source (flask and adapter made of Pyrex glass)

operated and relatively inexpensive. The leads from the H.F. Tester to the firing terminals may be of any convenient length, allowing remote-control firing from behind a suitable safety screen. The position of the platinum-gauze combustion basket in relation to the ancillary firing electrode is as shown in Fig. 2, so that the flame from the burning sample does not

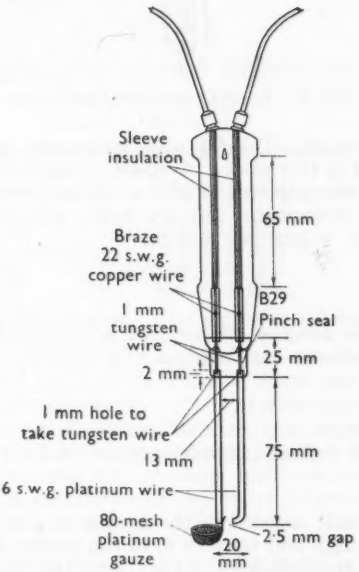

Fig. 2. Firing adapter

play on the platinum wire, which, by a cooling effect, may on occasion lead to the formation of unburnt carbon in the flask. It is important that the joint between the firing adapter and the combustion flask should show no signs of leakage.

Electrode assembly—The electrode assembly consists of a silver-wire indicator electrode and a silver wire in contact with a dilute solution of silver ions as reference. The electrode system is shown in Fig. 3, and is conveniently supported in a Perspex holder designed to fit into the top of the combustion flask and be retained by springs hooked to the lugs on the rim of the flask. A hole is drilled in this holder to permit the titrant-inlet tube from the titration syringe to be inserted into the flask. The reference-cell solution is prepared by adding 0·1 ml of 0·01 *N* silver nitrate to a mixture of 0·15 ml of *N* nitric acid and 30 ml of 1 per cent. potassium nitrate solution.

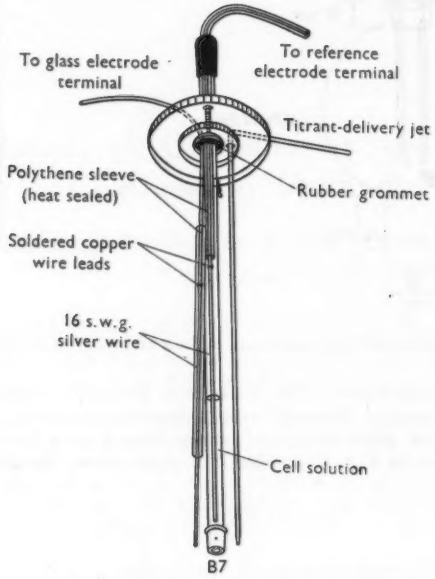

Fig. 3. Titration electrodes and holder

Full-scale automatic titrimeter—The full-scale automatic titrimeter that has been previously described[2,3] was used in this work. A 30-ml syringe was used to deliver the titrant, and the recorder sensitivity was adjusted to give a full-scale deflection of 400 mV registered by the pH meter. Other instrument settings were: injection-motor speed, 1 inch in 10 minutes; and chart speed, 1 inch per minute.

REAGENTS—

Sodium hydroxide, N.
Sodium hydrogen sulphite solution, 35 *per cent. w/v*—M.A.R. grade.
Hydrogen peroxide, 100-*volume*—AnalaR.
Silver nitrate, 0·01 N.
Nitric acid, N.
Methyl red indicator solution.
Sodium chloride—AnalaR sodium chloride was heated for 6 hours at 270° C before use.

PROCEDURE—

Weigh accurately a suitable amount of the sample to give 5 to 6 mg of chlorine (this weight should not exceed 30 mg), and transfer it to the centre of a small piece of Whatman No. 42 filter-paper weighing approximately 0·1 g. Fold the filter-paper so that the sample is completely enclosed, but before making the final fold, insert a small wick of cotton-wool.

Prepare the combustion vessel by washing the flask and adapter and heating the end of the platinum wire and the basket to red heat in a bunsen flame. Put 1 ml of *N* sodium hydroxide, 3 drops of 35 per cent. sodium hydrogen sulphite solution and 3 ml of water in the flask, and agitate it in a horizontal plane to wet the walls of the flask with the absorption solution. Fill the flask with oxygen and insert the stopper. Place the wrapped sample in the platinum basket,* and twist the cotton-wool wick so that it lies between the basket and the ancillary firing electrode. Remove the stopper from the flask, and replace it with the adapter containing the sample, seal the joint with a suitable amount of distilled water, and attach the retaining springs. Attach the electrical and earth leads to the adapter, and place the safety screen in front of the combustion vessel. Switch on the H.F. Tester to ignite the cotton-wool and sample, and allow to burn to completion. Set aside for 10 minutes, and then disconnect the electrical and earth leads from the adapter, and remove the springs. Disconnect the adapter from the combustion vessel, wash down the platinum wires and basket into the lower compartment of the combustion vessel, and rinse the sides of the flask to give a final volume of approximately 35 ml. Add 6 drops of 100-volume hydrogen peroxide to destroy any excess of sulphite, neutralise the solution with *N* nitric acid to methyl red indicator, and add 0·15 ml of nitric acid in excess.

Place a ¾-inch polythene-covered magnet in the combustion vessel, fit the electrode assembly, securing it by means of the springs provided, and place the flask on a magnetic stirrer. Titrate with 0·01 *N* silver nitrate until the titration record shows that the end-point has been passed. Carry out a blank combustion omitting only the sample, and titrate this at the same instrument settings. Finally, standardise the silver nitrate solution by carrying out a blank combustion with a known weight of sodium chloride (about 10 mg) added to the absorption solution in the combustion flask before the test. Titrate this solution under the same conditions as used for the sample and blank.

Deduce the end-points for all sample and standardisation titrations from the points of maximum inflexion on the titration curves. This point is conveniently found by means of a Perspex cursor inscribed with a series of concentric circles. The circle of most perfect fit is found for each side of the titration curve and the intersection of the line joining the centres of these circles cuts the titration curve at the required point of maximum inflexion. Correct each titre for the blank titre corresponding to the same end-point potential difference (this blank is only of the order of 0·07 minute titration time—about 0·05 ml), and hence calculate the chlorine content of the sample.

Results

Results obtained by applying the proposed procedure to known chlorine compounds are shown in Table I.

Table I

Determination of chlorine by proposed method

Compound	Chlorine found, %	Theoretical value, %
p-Chlorobenzoic acid	22·4, 22·4, 22·5	22·65
Benzylthiuronium chloride ..	17·5, 17·5, 17·5	17·49
Hexachlorobenzene	74·9, 74·9, 75·1	74·70
Poly(vinyl chloride)	56·7, 57·1, 56·9	56·6†

† Result obtained by micro Carius and peroxide-fusion methods.

We have found that, in practice, the apparatus is extremely simple to use; for serial determinations we recommend that four combustion flasks and two firing adapters be provided. It is suggested that the apparatus may have wide application in the determination of other elements by the oxygen flask combustion method and, moreover, be of value in the qualitative detection of elements in organic compounds.

Since this work was completed our attention has been drawn to an alternative form of adapter designed by Martin and Deveraux[4] for electrical ignition in the Schöniger oxygen flask method.

* If the platinum gauze has fused and developed a hole during previous combustions, it should be covered up with a fresh piece of gauze for the next test. When the gauze can no longer be used, it is returned to the makers for recovery. The cost of an individual test is negligible.

We thank Mr. K. R. Clarke for his collaboration in the development of the remote-control firing unit and Miss M. Clark for her assistance in this investigation.

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The Determination of Microgram Amounts of Fluorine by Diffusion

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The determination of small amounts of fluorine in biological samples without distillation is described. The method involves the collection of fluorine by diffusion in a polythene bottle, absorption by alkali-treated filter-paper and subsequent titration with thorium nitrate solution in the presence of alizarin sulphonate. The method is simple and uncomplicated by the problems associated with conventional distillation techniques.

The micro-determination of fluorine by a diffusion technique was first described by Singer and Armstrong,[1] but appears to have attracted little attention. A re-appraisal of this method was prompted by the need for determining fluorine in single small leaves of *Dichapetalum cymosum* (gifblaar), a South African plant containing fluorine in the form of a fluoroacetate.

The procedure proposed here for collecting hydrofluoric acid is simpler than that proposed by Singer and Armstrong and permits the determination of as little as 1 μg of fluorine. The sample is suitably treated to convert all forms of fluorine to inorganic fluoride, which is then absorbed by filter-paper impregnated with potassium hydroxide after diffusion as hydrofluoric acid. The determination of fluoride by titration with thorium nitrate solution has been adapted from that described previously.[2]

Method

Reagents—

Whenever possible, all materials should be of analytical-reagent grade.

Thorium nitrate, 0·001 *N*—Dissolve 138 mg of $Th(NO_3)_4.4H_2O$ or 147 mg of $Th(NO_3)_4.6H_2O$ in water, and dilute to 1 litre.

Monochloroacetate buffer solution, pH 3·0—Dissolve 22·7 g of monochloroacetic acid in 800 ml of water, slowly add 120 ml of *N* sodium hydroxide, and dilute with water to 1 litre.

Buffered alizarin sulphonate solution—To 20 ml of a 0·01 per cent. w/v solution of alizarin sulphonate in water add 10 ml of the monochloroacetate buffer solution and then 5 ml of 0·001 *N* thorium nitrate, and dilute with water to 100 ml.

Standard fluoride solution—Dissolve 22·105 mg of sodium fluoride in water, and dilute to 1 litre. This solution contains 10 μg of fluoride ion per ml.

Preparation of sample—

Biological specimens are dried and ground, and a 1-g sample is treated in a small platinum dish with 2 ml of *N* potassium hydroxide in 50 per cent. aqueous propyl alcohol and then dried at 100° C. When dry, 1 ml of a 10 per cent. w/v solution of calcium nitrate in *n*-propyl alcohol is added, and the contents of the dish are evaporated to dryness. The dish is placed in a muffle furnace, and the temperature is slowly increased to 400° C and maintained at this value for several hours. (It is usually convenient to leave the dish in the furnace overnight.)

The carbonaceous residue is carefully powdered, mixed with 100 mg of sodium peroxide and re-heated in the muffle furnace for 3 to 4 hours. The ash is transferred to a 10-ml graduated tube and treated with *N* perchloric acid until it has dissolved. The pH of the solution is adjusted to about 6 by careful addition of 0·5 *N* potassium hydroxide, and the volume is reduced to between 2·0 and 2·5 ml by blowing compressed air on to the surface of the solution, the tube being immersed in water at about 70° C. The pH of the solution is then re-adjusted to 3 with 0·2 *N* perchloric acid, and the volume is made to 2·5 ml. Any insoluble material is separated by centrifugation.

Specimens of fluoro compounds (2 to 5 mg) are weighed into a Parr-type bomb and treated with 1 ml of *N* potassium hydroxide in 50 per cent. aqueous propyl alcohol and with 1 ml of a 10 per cent. w/v solution of calcium nitrate in *n*-propyl alcohol. The solvents can be removed by means of a gentle stream of compressed air, with the bomb chamber standing on a hot-plate set at low heat, or by slow evaporation to dryness in an oven at 100° C. To the dried preparation, 100 to 200 mg of sodium peroxide or 50 to 100 mg of sodium metal cut into very small pieces are added. The bomb is sealed and heated at 500° C for 2 hours in the muffle furnace, in which it is also allowed to cool. The digest in the bomb, which should be a clean white solid, is dissolved in *N* perchloric acid, and the solution is diluted to 25 ml with water after adjustment to pH 3 as before. A blank solution of the reagents used in either of the procedures described above is similarly prepared.

DIFFUSION OF HYDROFLUORIC ACID—

The collection of the fluorine by diffusion as hydrofluoric acid is carried out in 20-ml polythene bottles with screw caps (obtainable from A. R. Horwell, Cricklewood, London, N.W.2). These were chosen because the cap could be fitted with an adapter of polythene tubing to hold a cylinder of filter-paper impregnated with potassium hydroxide solution for absorbing the hydrofluoric acid. The assembly is shown in Fig. 1. A strip of Whatman No. 541 filter-paper (3 cm × 2 cm) is wrapped round a short length of glass rod (15 mm × 7 mm) that just fits inside the polythene tubing (25 mm × 7 mm internal diameter). The glass rod and filter-paper are pushed into the tubing so that 2 cm of the paper are left exposed, and the rod is then pushed entirely into the adapter, holding the filter-paper firmly in position. The free end of the polythene tubing is then fitted into the well of the screw-cap.

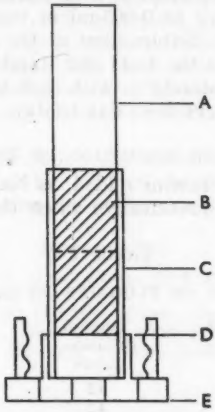

Fig. 1. Sectional diagram of polythene-cap assembly showing filter-paper in position: A, cylinder of filter-paper; B, glass rod; C, polythene tubing; D, well of cap; E, bottle cap

A 1-ml portion of the solution to be tested is placed, by pipette, in the diffusion bottle, and 1 drop (0·03 ml) of 3 *N* potassium hydroxide (containing 1 ml of a 0·5 per cent. w/v ethanolic solution of phenolphthalein per 100 ml) is placed on the exposed section of the filter-paper. By pipette, 2 ml of 72 per cent. w/v perchloric acid are placed in the bottle, ensuring that none touches the inside of the neck. The cap containing the treated filter-paper

is immediately placed on the bottle and is tightened with a pair of pliers. It is advisable to set up the bottles in duplicate. Four other bottles (diffusion blanks) are prepared, each containing 1 ml of water instead of test solution, and extracts of the filter-papers from these bottles are used in titrating the standard fluoride solution with 0·001 *N* thorium nitrate. The bottles are placed in an oven at 60° C for 24 hours.

When a filter-paper is removed from its bottle, it will be noticed that the area containing potassium hydroxide solution is pale yellow and its edge red. This area is cut off, and extracted with 3 ml of water in a 5-ml graduated tube placed in a boiling-water bath for 2 minutes. When cool, the solution is made just acid to phenolphthalein with 0·1 *N* perchloric acid and then diluted to 5 ml with water. The extracts of the four diffusion blanks, after similar preparation, are combined.

DETERMINATION OF FLUORIDE—

Adjustment of pH—The pH of the solution to be titrated with 0·001 *N* thorium nitrate is adjusted to 3 by titration with 0·04 *N* perchloric acid, bromophenol blue being used as indicator. The colour standard for pH 3 is prepared by placing 2 ml of water and 0·02 ml each of a 0·04 per cent. w/v aqueous solution of bromophenol blue and 0·04 *N* perchloric acid in a 5·0-cm × 1·5-cm specimen tube. In a similar tube, 2 ml of test solution are titrated with 0·04 *N* perchloric acid, with 0·02 ml of the bromophenol blue solution as indicator, until the colour is the same as that of the standard. The volume of 0·04 *N* perchloric acid used is noted, and this volume is added to another 2-ml portion of test solution, but without the indicator solution.

Volumes of standard fluoride solution containing 1·0, 3·0 and 5·0 μg of fluoride are added to 2-ml portions of the combined diffusion blanks after the pH has been adjusted to 3 as described above.

Titration—To each of the adjusted test, blank and standard solutions is added a 2-ml portion of buffered alizarin sulphonate solution, the solutions are mixed by means of a few air bubbles from a fine Pasteur pipette and then set aside for about 5 minutes. (The solutions containing fluoride rapidly become pale yellow.) The contents of each tube are titrated with 0·001 *N* thorium nitrate, added in 0·02-ml increments from a 1-ml microburette, until the yellow colour changes to a shell-pink that matches the colour of a blank solution consisting of 2 ml of water, 0·02 ml of 0·04 *N* perchloric acid and 2 ml of buffered alizarin sulphonate solution. The end-point is sensitive to 0·005 ml of titrant and is easily seen, preferably against a white matt background. Subtraction of the titre for the diffusion blank gives the value for the fluorine titrated in the tests and standards.

It is advisable to titrate fresh standards with each set of determinations, because small variations in the titration values occur from day to day.

RESULTS AND DISCUSSION OF THE METHOD

Table I shows the recovery of fluorine added, as NaF, to the diffusion bottles. It can be seen that recovery is virtually quantitative when the diffusion is carried out at 60° C for 24 hours.

TABLE I

RECOVERY OF FLUORINE BY DIFFUSION

Amount of fluorine added, μg	Temperature of diffusion, °C	Time of diffusion, hours	Amount of fluorine found, μg	Recovery, %
2·50	20	42	0·58	22
5·00	20	42	3·30	66
2·50	37	42	1·67	67
5·00	37	42	3·45	69
2·50	50	20	2·35	94
5·00	50	20	4·37	87·5
2·50	60	18	2·50	100
5·00	60	18	4·84	96·6
2·50	60	24	2·50	100
5·00	60	24	4·93	98·6

The determination of small amounts of fluorine presents many problems, and no attempt is made here to discuss in detail all aspects of the subject. The preparation of samples,

particularly of biological material, without incurring loss of fluorine is not easy, and, after the fluorine has been converted to inorganic fluoride, it must be separated from substances that interfere with the reagents used in the final stages. The conventional procedure is distillation of the fluorine as hydrofluorosilicic acid.[3] Recently, however, in convincing work, Fox and Jackson[4] have reported that fluorine is in fact distilled as hydrofluoric acid. Numerous variations of this process have been recommended, including a technique for the recovery of less than 10 μg of fluorine in an apparatus maintained at 146° C by using a reflux chamber containing *sym.*-tetrachloroethane.[5] Nevertheless, in my experience, the recovery of a few micrograms of fluorine is most difficult, and results rarely approach present-day standards of reproducibility. The bleaching action of some distillates on the alizarin - thorium complex is difficult to prevent, and the need to collect large volumes of distillate to ensure complete recovery is time-consuming and tedious. The proposed method is simple and requires no unusual apparatus; many determinations can be made in a short time.

For the collection of hydrofluoric acid in the diffusion bottle, Singer and Armstrong[1] proposed the use of a strip of polythene, one side of which was roughened and treated with 0·5 *N* sodium hydroxide. This was found to be less convenient than the use of small cylinders of filter-paper, Whatman No. 541 being chosen because of its resistance to concentrated solutions of alkali. It was also found that a concentration of sodium hydroxide much greater than 0·5 *N* was required for complete recovery. Maintenance of the diffusion vessel in an ice-bath during transference and freezing the sample[1] were found to be unnecessary in this laboratory.

Temperature, time and the volume of liquid in the diffusion bottle are important factors. Complete recovery of 2·5 and 5·0 μg of fluorine was not achieved until diffusion was carried out at 60° C for 24 hours. The observations of Singer and Armstrong[1] on the effect of the total volume of liquid in the bottle were confirmed; under the experimental conditions, 4 ml of liquid, with a depth of 0·5 cm, is the maximum volume permissible for complete recovery. In larger bottles, the hydrofluoric acid becomes widely dispersed, and its absorption by the potassium hydroxide solution is slower; in this respect, a 20-ml bottle is preferable to the 60-ml bottle used by Singer and Armstrong.[1] Recoveries were low when glass diffusion bottles were tried. With the proposed method, no interference with the thorium nitrate titration has been encountered, but Singer and Armstrong reported only 87 per cent. recovery of 20 μg of fluorine when 10 mg of glucose were also present. High concentrations of chloride or bromide could inhibit absorption of hydrofluoric acid by neutralising the potassium hydroxide, but the concentrations needed to effect this seldom arise, except in a determination of free fluoride in untreated body fluids. The most serious interfering substance with other procedures—phosphate—is completely innocuous. The proposed method is therefore a means of separating small amounts of fluorine without the tedium and uncertainties of distillation.

I acknowledge with much appreciation the constant interest shown by Sir Rudolph Peters, F.R.S., and I am grateful to Dr. G. Neil Jenkins, King's College, Newcastle, for drawing my attention to the determination of fluorine by diffusion.

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The Determination of Microgram Amounts of Nitrate in Chromic Acid

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The determination of microgram amounts of nitrate-N in chromic acid (chromium trioxide) is described. Chromium is reduced by ethanol, and nitrate is then reduced to ammonia by Devarda's alloy in alkaline solution in the presence of a precipitate of chromium hydroxide. The ammonia formed is separated by steam-distillation and is determined from the intensity of the colour developed with Nessler's reagent. Adsorption of nitrate on the precipitate, which causes losses in methods involving separation of chromium by precipitation and filtration, does not affect the reduction to ammonia. Reproducibility at a level of 10 μg of nitrogen is ± 5 per cent.; at the 150-μg level, it is ± 1 per cent. For a 10-g sample, this is equivalent to ± 5 per cent. of nitrogen at the 1 p.p.m. level and to ± 1 per cent. at the 15 p.p.m. level.

THERE is a requirement at these laboratories for high-purity electro-deposited chromium metal containing a minimum amount of nitrogen. This has led to persistent efforts to prevent contamination of the electrolyte bath by nitrogen-containing impurities, which could produce atomic nitrogen by cathodic reduction. The most likely impurity in the bath is nitrate ion, and the considerations described below indicate that its source is the chromic acid used.

The bath consists of 300 g of chromic acid, 7 g of concentrated sulphuric acid and distilled water to make 1 litre. The water contains less than 0·001 p.p.m. of nitrate, so that, although large amounts are used, its contribution to the nitrate content of the bath is negligible. Sulphuric acid contributes little nitrate, for, although analytical-reagent grade acid may contain a maximum of 0·2 p.p.m. of nitrate,[1] its content in the bath is small and additions are made infrequently. With chromic acid, however, the position is different. The continuous process used for the electro-deposition of chromium involves addition of 2000 g of chromic acid each 24 hours for an average yield of 780 g of chromium metal. Analytical-reagent grade chromic acid may contain a maximum of 40 p.p.m. of nitrate,[2] and, if material containing this amount of nitrate is used, the metal obtained on electrolysis could contain up to 23 p.p.m. of nitrogen. It has therefore been important to use chromic acid containing a negligible amount of nitrate and to check the nitrate content of each batch used by a method of adequate accuracy and sensitivity.

Two methods[3,4] for determining nitrate in chromic acid were found to be unsatisfactory because of the extensive loss of nitrate occurring during the separation step for the removal of chromium by precipitation. For example, a decrease in sensitivity from 0·4 to 20 mg of nitric acid per litre was reported[3] when chromium was removed from solution by precipitation as barium chromate. We confirmed this decrease, and it consequently became necessary to develop a method in which interference by chromium was avoided and which had the required accuracy and sensitivity.

Experimental

Preliminary experiments—

The most likely approach appeared to be one based on reduction of nitrate to ammonia by Devarda's alloy in alkaline solution, separation of the ammonia by steam-distillation, and its determination from the intensity of the colour developed with Nessler's reagent. Traces of ammonia in the initial solutions would be removed by boiling the alkaline solution before Devarda's alloy was added. When 100 μg of nitrate-N, present as potassium nitrate, were so treated, the optical-density readings were identical with those obtained by treating 100 μg of ammonia-N, present as ammonium chloride, directly with Nessler's reagent. When 5 g of chromic acid were added to the solution of potassium nitrate, however, reduction to ammonia by Devarda's alloy was completely inhibited. This indicated that chromium must be reduced from the sexavalent state or removed from the solution before the nitrate was reduced in alkaline solution.

When ethanol was used as reductant, it was found that quantitative reduction and recovery of 150 μg of nitrate-N could be achieved in presence of a precipitate of chromium hydroxide derived from up to 10 g of chromic acid. Unsuccessful attempts were made to find reagents that would reduce and then form a complex with the chromium so as to provide cleaner operating conditions and more certain recovery of ammonia. For example, citric and tartaric acids reduced and formed complexes with the chromium, tartaric acid being more effective. Volatile by-products were formed, however, when tartaric acid was used, even when the acid was added only to form a complex with the chromium after it had been reduced by ethanol. These by-products interfered with the development of colour between the ammonia in the distillate and Nessler's reagent. Consequently, a procedure based on the use of ethanol as reducing agent was developed to cover the range 10 to 150 μg of nitrate-N.

To ensure complete recovery of the ammonia formed it was found to be necessary to collect 100 ml of distillate, to which were added 2 ml of Nessler's reagent for colour development. Maximum colour intensity was developed by setting the solution aside for 30 minutes at 25° C.[5,6] Optical-density measurements were made against water at 4100 A with a Beckman DU spectrophotometer. A stable background colour was produced by adding Nessler's reagent to ammonia-free distilled water. In solutions containing less than 20 μg of nitrogen, however, colour development was variable and slow, maximum intensity taking up to several hours to develop. This difficulty was overcome by adding 20 μg of nitrogen, as ammonium chloride solution, to blank distillates and to distillates expected to have such low contents of nitrogen.

Method

Reagents—

Distilled water, ammonia-free.

Devarda's alloy—Nitrogen content should be negligible.

Ethanol, absolute—Distil, and discard the first and last 15 per cent. of distillate.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

Nessler's reagent solution—Dissolve 50 g of potassium iodide in the minimum amount of cold water, and add a saturated solution of mercuric chloride until a slight precipitate persists. Add 400 ml of potassium hydroxide solution (50 per cent. w/v), dilute to 1 litre, allow to settle, and use the supernatant liquid.

Sodium hydroxide solution, 50 per cent. w/v—Dissolve 500 g of sodium hydroxide in 1100 ml of water, and digest overnight with Devarda's alloy. Boil until the volume has decreased by approximately 200 ml, cool, and dilute to 1 litre with ammonia-free distilled water.

Standard ammonium chloride solution—Dissolve 0·0382 g of ammonium chloride in distilled water, and dilute to a litre.

1 ml ≡ 10 μg of nitrogen.

Preparation of calibration graph—

Dilute portions of standard ammonium chloride solution containing from 20 to 200 μg of nitrogen to 100 ml with distilled water, and place in a water bath maintained at 25° C. To each solution add 2·0 ml of Nessler's reagent solution, mix, and set aside for 30 minutes. Measure the optical density of each solution against water in 2-cm cells at 4100 A, and plot a graph of optical density against nitrogen content. Optical-density readings obtained during preparation of a calibration graph are shown in Table I.

Procedure—

Transfer 100 ml of an aqueous solution containing a maximum of 10 g of chromic acid to a 500-ml conical beaker, add 30 ml of hydrochloric acid, sp.gr. 1·18, and 10 ml of ethanol, and warm for 10 minutes. Add 5 ml of ethanol, set aside for 5 minutes, and boil vigorously for 15 minutes to remove the excess of ethanol. Transfer to a 700-ml distillation flask, add 45 ml of 50 per cent. sodium hydroxide solution, attach the flask to a distillation apparatus, and steam-distil until approximately 150 ml of distillate have been collected. Discard the distillate, remove the flask from the distillation apparatus, and allow to cool to room temperature. Place 1 g of Devarda's alloy in the flask, re-attach to the distillation apparatus, and steam-distil until 95 ml of distillate have been collected in a 100-ml calibrated flask. Place

the flask in a water bath maintained at 25° C, dilute to the mark with distilled water, add 2·0 ml of Nessler's reagent solution, and mix. After 30 minutes, measure the optical density of the solution against water at 4100 A in 2-cm cells.

Determine the nitrogen content of distillates containing between 20 and 150 μg of nitrogen by reference to the calibration graph. For the blank solution and distillates containing less than 20 μg of nitrogen, add 2·0 ml of standard ammonium chloride solution before dilution to 100 ml, and then continue as described above. Calculate the nitrate-N content of the sample by subtracting the appropriate blank value from the total amount of nitrogen determined.

Table I

Optical-density readings for ammonia - Nessler reagent complex

Each result is the mean of at least three measurements at 25° C. The volume of solution used was 100 ml, and 2·0 ml of Nessler's reagent solution were added

Nitrogen equivalent of ammonium chloride solution used, μg	Optical density
0	0·031 ± 0·001
20	0·115 ± 0·001
30	0·157 ± 0·001
50	0·225 ± 0·002
75	0·322 ± 0·002
100	0·418 ± 0·002
125	0·525 ± 0·002
150	0·631 ± 0·002
200	0·841 ± 0·002

Results

Portions of a standard solution of potassium nitrate (10 μg of nitrate-N per ml) containing 10 to 150 μg of nitrogen were added to 100-ml portions of solutions containing 10 g of nitrate-free chromic acid. The nitrate content of each solution was then determined in triplicate by the proposed procedure. The blank value was approximately 1·5 μg of nitrogen; the results are shown in Table II.

Table II

Recovery of nitrate-N added to nitrate-free chromic acid

Nitrogen equivalent of added potassium nitrate solution, μg	Nitrogen recovered, μg
10	10·2, 9·8, 9·5
20	20·2, 20·0, 20·0
30	30·7, 30·7, 30·3
50	49·8, 49·5, 49·2
100	100·5, 100·3, 99·8
125	125·5, 125·5, 125·5
150	150·5, 150·0, 149·5

Several samples of analytical-reagent and commercial grade chromic acid were also analysed in triplicate; the results are shown in Table III.

Discussion of the method

The results in Table II show that the presence of the large precipitate of chromium hydroxide formed by adding sodium hydroxide to the solution containing chromic and nitrate ions has no effect on the reduction of nitrate by Devarda's alloy. It is therefore not necessary to remove chromium before determining nitrate.

Solutions of the ammonia - Nessler reagent complex formed under the conditions described obey Beer's law for amounts of nitrogen between 100 and 200 μg, as shown by the results in Table I. Between 20 and 100 μg of nitrogen, optical densities are greater than would be expected from the slope of the graph between 100 and 200 μg of nitrogen, the extent of the deviation increasing progressively as the amount of nitrogen present decreases.

The reproducibility of optical-density readings during the recovery experiments with potassium nitrate solution was equal to that obtained during calibration with standard

ammonium chloride solution. For a sample weighing 10 g, this is equivalent to ±5 per cent. of nitrogen at the 1 p.p.m. level and to ±1 per cent. of nitrogen at the 15 p.p.m. level.

TABLE III

NITRATE-N FOUND IN CHROMIC ACID

Origin of chromic acid	Sample No.	Weight of sample, g	Nitrate-N found, p.p.m.
Analytical-reagent grade material—			
English	1	10	12·1, 12·2, 12·2
	2	2	60·3, 60·4, 60·8
	3	5	17·3, 17·3, 17·4
	4	4	26·1, 26·1, 26·2
	5	10	<1
	6	10	
Commercial-grade material—			
Australian	7	10	<1
German	8	10	

Although the accuracy and sensitivity are adequate, the sensitivity could be increased up to a maximum of 100-fold by a modification to the method. This would involve evaporation of the distillate to small volume after acidification, and then treatment by a micro-diffusion technique,[7] so that the determination could be carried out in 1 ml of solution. The presence of traces of ammonia in the atmosphere of our laboratory made it impracticable to extend the method along these lines.

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A Source of Serious Error in the Determination of Nitrates by the Phenoldisulphonic Acid Method and its Remedy

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In the presence of similar amounts of ammonium ions, losses of nitrate-N can amount to as much as 50 per cent. Such losses can be completely prevented by preliminary evaporation in the presence of a small amount of potassium hydroxide. A mechanism for the loss is suggested.

THE phenoldisulphonic acid method for determining nitrate-N was introduced by Sprengel[1] and, since its investigation by Chamot and his co-workers,[2,3,4] has become firmly established in many laboratories, more especially those engaged in the analysis of water. The sensitivity of phenoldisulphonic acid and the fact that it forms a definite compound with nitrate ions give it distinct advantages over reagents such as diphenylamine and brucine, which depend

on the oxidising properties of nitrates and are therefore severely limited in their use for quantitative work. We believe that this paper will enhance the value of the phenoldisulphonic acid method and extend its usefulness.

Experimental

During work on plant-water cultures we wished to investigate the uptake of ammonia-N and nitrate-N, the element being supplied solely as ammonium nitrate. The photometer was calibrated for ammonia by using Nessler's reagent and pure ammonium sulphate and for nitrates by using the phenoldisulphonic acid method and pure potassium nitrate (the phenoldisulphonic acid used was of laboratory-reagent grade, obtained from the British Drug Houses Ltd.). The calibration was then checked by determining both forms of nitrogen in the water culture, to which carefully weighed amounts of ammonium nitrate had been added. Chlorides and nitrites were absent from this culture solution, and interference from calcium and magnesium in the Nessler determination was completely prevented by using Rochelle salt. Since ammonium nitrate was used, both forms of nitrogen, as nitrogen, should have been present in equal amounts. The result found for ammonia-N entirely agreed with the expected amount, but numerous determinations of nitrate-N showed maximum recoveries of about 50 per cent. and more often less. The presence of ammonium ions was suspected of causing the low recoveries, and support for this was obtained when both forms of nitrogen were simultaneously determined in a standard solution of AnalaR ammonium nitrate. Recoveries were complete when the Nessler reagent was used, but were again about 50 per cent. or less with the phenoldisulphonic acid reagent solution. Removal of the ammonium ion was then attempted.

Since the phenoldisulphonic acid method involves preliminary evaporation to dryness of the nitrate-containing sample, it was decided to try the effect of evaporating in presence of 1 or more drops of the 30 per cent. solution of potassium hydroxide used later for developing the yellow colour. Replicate experiments showed that the addition of 2 drops (0·10 ml) of this potassium hydroxide solution to a 10-ml portion of the standard ammonium nitrate solution immediately before evaporation resulted in complete recovery of nitrate. Similar experiments with the water-culture solution were also completely satisfactory; both forms of nitrogen were recovered in equal amounts, and these amounts agreed with the amount of ammonium nitrate added. The same was true when standard solutions of potassium nitrate were used.

By the time the 10-ml sample has been evaporated to dryness, the added potassium hydroxide has been converted to potassium carbonate. We are aware that Chamot, Pratt and Redfield[3] referred to losses of nitrate in the presence of carbonates when the residue from evaporation was treated with the phenoldisulphonic acid reagent solution, the loss being mechanical and caused by effervescence resulting from the addition of acid. They showed that such losses were "insignificant," except when "the alkalinity of the water is very high or the amount of nitrate present is very low." We have never encountered any loss from standard solutions of potassium nitrate or ammonium nitrate when the amount and concentration of potassium hydroxide stated above were used. We think it may be important in this connection that the phenoldisulphonic acid reagent solution be not added until the residue from evaporation has been allowed to cool to room temperature. The amount of effervescence then occurring is at a minimum.

We have been unable to find any reference to this serious interference from ammonium ions. Snell and Snell[5] recommended that the nitrate solution be made "alkaline," and Allport[6] that it be "faintly alkaline or neutral," but it seems that the interference was unknown to Chamot, Pratt and Redfield, who, for water analysis,[4] advise the addition of very dilute sulphuric acid "to not quite neutralise all the alkalinity"; this, however, was as a precaution against loss from carbonates. The interference may have been missed, because it is possible, at least in natural fluids, that the oxidised and reduced forms of inorganic nitrogen do not often occur together, or, if they do, then not in more or less equal amounts, such as might obtain in a water-culture solution.

Possible mechanism of the loss

We have not attempted to investigate the mechanism of this loss of nitrate-N, but on chemical grounds it seems likely that it occurs via nitramide, NH_2NO_2. This isomer of hyponitrous acid can be regarded as a derivative of nitric acid, $HONO_2$, in which the –O H

group has been replaced by an $-NH_2$ group.[7] Such a replacement might be expected to take place when the phenoldisulphonic reagent solution (which contains fuming sulphuric acid) is added to the evaporated residue of ammonium and nitrate ions. Nitramide decomposes according to the equation—

$$NH_2NO_2 = N_2O + H_2O.$$

This reaction occurs slowly in aqueous and acid solution, but is very rapid in alkaline solution, and the nitrated phenoldisulphonic acid reagent must be made alkaline in order to develop the yellow colour.

In connection with the choice of alkali for this purpose, we have always used a 30 per cent. solution of potassium hydroxide, not only because Chamot and his co-workers[3] found this better than the equivalent concentration of sodium or ammonium hydroxide, but also because we did not wish to have ammonia fumes in the laboratory when determining ammonia-N. The results reported by Chamot and his co-workers[3] all show loss of nitrate-N when ammonium hydroxide was used, but since this reagent was otherwise "undesirable," they did not investigate the matter further. Perhaps here again, loss via nitramide might be involved.

To judge from more recent literature, it seems that most British analysts recommend the use of ammonium hydroxide, presumably on the grounds that, when chlorides are present and have to be removed by adding silver sulphate solution, potassium hydroxide is liable to produce an undesirable tint with any residual silver salt, so making it difficult to match the yellow colour. Although not disputing this, we would strongly recommend that, when chlorides are absent or present only in such small amounts that their removal is unnecessary, potassium hydroxide should be used. In such circumstances, our results have been excellent, but this was not so when ammonium hydroxide was used.

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The Colorimetric Determination of Vanadium with Benzoylphenylhydroxylamine

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A method is described for separating and determining small amounts of vanadium. The complex formed when benzoylphenylhydroxylamine is added to solutions of quinquivalent vanadium in 5 to 9 *M* hydrochloric acid is extracted by chloroform and the optical density of the resulting coloured solution is measured. The reaction is extremely selective; commonly associated metals do not interfere and it may be used for the rapid determination of vanadium in steels and ores.

Various organic reagents have been recommended for the colorimetric determination of vanadium during the past 15 years[1 to 7]; most of these are subject to interferences from commonly associated elements owing to their masking action or to reaction with the reagent. Cupferron has been used by Bertrand[8] to determine vanadium in vegetables by extracting the complex with chloroform, but preliminary separation of copper, iron, titanium and cerium was necessary. Willard, Martin and Feltham[9] used this same compound to separate

vanadium from copper, interference from copper being overcome by means of ethylenediaminetetra-acetic acid; the procedure required precipitation at controlled pH and temperature before solution in acetone and measurement of the optical density. Benzoylphenylhydroxylamine, in which the benzoyl group has replaced the nitroso group in cupferron, is more sensitive to hydrogen ion and is therefore more selective in its action[10,11]; it yields precipitates, in solutions containing more than 1 per cent. of concentrated hydrochloric acid, only with tin, titanium, zirconium and vanadium.[10,12] The possibility of thus overcoming many interferences by the use of benzoylphenylhydroxylamine was attractive. Further, as the benzoylphenylhydroxylamine - vanadium complex is soluble in organic solvents, the removal of masking interferences appeared possible. Shome[13] used this reagent for determining vanadium colorimetrically, but, under the conditions of reaction, many ions interfered and close control of pH (1·9 to 2·8) was necessary.

Experimental

From dilute solutions of vanadate in sulphuric acid a brown precipitate is obtained when an alcoholic solution of benzoylphenylhydroxylamine is added; this precipitate is readily extracted by chloroform to give an orange-yellow colour with maximum absorption at 450 mμ and a molecular extinction coefficient of 3700. The colour is similar to that obtained by Shome[13] and is stable, but reproducibility is dependent on close control of pH.

In hydrochloric acid solutions, a purple solution and a precipitate are formed; extraction with chloroform gives a purple-coloured extract with maximum absorption at 530 mμ and a molecular extinction coefficient of 4490. The colour developed in aqueous solution fades rapidly with increasing hydrochloric acid concentration and does not reappear on further addition of reagent; with a solution of the reagent in chloroform, however, stable extracts are obtained from solutions 5 to 9 *M* in hydrochloric acid. The orange-coloured extracts resulting from solutions of vanadate in sulphuric acid immediately become purple when further acidified with hydrochloric acid and shaken.

No colour or precipitate is obtained when benzoylphenylhydroxylamine is added to solutions of quadrivalent vanadium, and the vanadium must be present in the quinquivalent state.

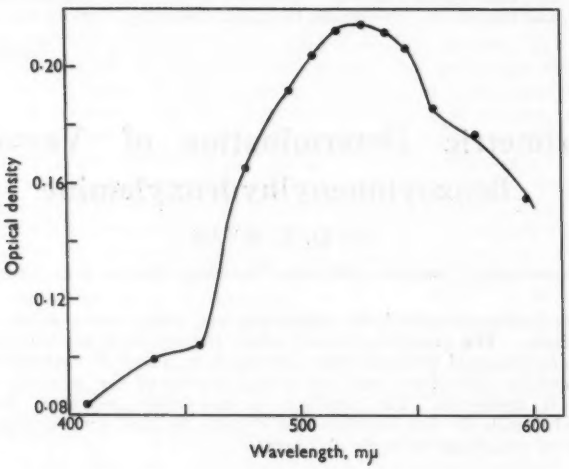

Fig. 1. Absorption curve for solution containing complex (0·123 mg of vanadium) in 50 ml of chloroform

The absorption curve for a solution containing 0·123 mg of vanadium (2·46 μg per ml) is shown in Fig. 1. The results were obtained with a Hilger spectrophotometer; this instrument, at a wavelength of 530 mμ, was used to obtain most of the figures recorded, although

excellent results were also obtained with a Spekker photo-electric absorptiometer and an Ilford No. 605 filter. The concentration limits that can be reasonably handled by the proposed procedure are 0·03 to 0·4 mg of vanadium.

Method

Reagents—

N-Benzoylphenylhydroxylamine reagent solution—Prepare the reagent as previously described,[14] and recrystallise from hot water; the compound can be purchased from the Eastman Kodak Company. Prepare a solution by dissolving 0·5 g in 100 ml of analytical-reagent grade chloroform; this solution is colourless and, if stored in a dark bottle, is stable for some months.

Standard vanadium solution—Dissolve sodium vanadate in distilled water containing 1 per cent. of concentrated sulphuric acid, and then heat. Filter the resulting solution, dilute to a known volume, and determine the vanadium content gravimetrically by means of cupferron. Obtain suitable concentrations by diluting this standard solution.

Procedure—

Prepare a solution containing between 0·03 and 0·4 mg of vanadium in the quinquivalent state in 5 to 9 *M* hydrochloric acid. Transfer the solution or an aliquot containing an amount of vanadium within the above limits to a separating funnel, add 2 ml of benzoylphenylhydroxylamine reagent solution for each 0·1 mg of vanadium expected to be present, and dilute the organic layer to about 10 ml with chloroform. Shake well, allow the phases to separate, and remove the organic phase to a 50-ml calibrated flask. Extract the aqueous layer again with 10 ml of chloroform. Dilute the combined extracts to the mark with chloroform, and measure the optical density at 530 mμ in a 1-cm cell of a spectrophotometer or of a Spekker absorptiometer, an Ilford No. 605 filter being used.

Results

The optical densities obtained under various conditions are shown in Table I. These results are for 0·123 mg of vanadium, 2·0 ml of reagent solution and a 1-cm cell; the volumes of solutions before extraction were approximately 20 ml for each of these samples.

Table I

Optical densities of solutions containing 0·123 mg of vanadium under various conditions

	Condition	Optical density
(1)	5·0 ml of hydrochloric acid present	0·190
(2)	7·5 ml of hydrochloric acid present	0·216
(3)	10·0 ml of hydrochloric acid present	0·218
(4)	13·0 ml of hydrochloric acid present	0·218
(5)	As in (3) + 1·0 ml of concentrated sulphuric acid	0·220
(6)	As in (3) + 0·5 ml of concentrated nitric acid	0·225
(7)	As in (3), but with only 1·0 ml of reagent solution	0·180
(8)	As in (3), but with 3 ml of reagent solution	0·218
(9)	As in (3), but with 2·0 g of sodium chloride added	0·218
(10)	As in (3), but with 1 g of sodium tetraborate added	0·215
(11)	As in (3), but with 1 g of sodium tetraborate + 1 g of sodium sulphate added	0·216
(12)	1 g of sodium tetraborate + 1 g of sodium carbonate neutralised with sulphuric acid + hydrochloric acid as in (3)	0·215

The results show that vanadium can be readily separated and determined from solutions 5 to 9 *M* in hydrochloric acid by this procedure and that high salt concentrations cause no difficulty. Higher concentrations of sulphuric acid are readily tolerated, but nitric acid, if the concentration becomes too high, reacts with the reagent to give a yellow colour and must be controlled.

Optical density - concentration graphs were plotted; they showed that the coloured solution obeys Beer's law over the range applicable for a 1-cm cell. The optical density of a typical sample changed from 0·218 to 0·212 when the solution was kept in daylight in a closed container for 4 hours.

A study of the ratio of reagent to vanadium in the complex was carried out by the method of continuous variations and showed that two molecules of reagent are combined with one atom of vanadium; a graphical plot of optical density against gram-atoms of vanadium/gram-atoms of vanadium *plus* gram-molecules of benzylphenylhydroxylamine gave a well defined maximum at 0·33. In the colorimetric determination, however, an excess of reagent is necessary for complete colour development.

Reactions

The reactions of benzoylphenylhydroxylamine and of other N-acyl substituted phenylhydroxylamines have been described previously[10,11,12]; in solutions containing more than 1 per cent. of concentrated hydrochloric acid only tin, zirconium, titanium and vanadium react to give precipitates. Dichromate and permanganate are reduced in acid solution, a yellow to brown extract being obtained with chloroform; oxidising agents therefore interfere by reacting with the reagent and give a high result for the vanadium content. Such interferences are readily avoided, however, by treatment with ferrous iron, and the procedure can be easily modified to overcome these interferences. Results obtained in the presence of ions commonly causing difficulty in the determination of vanadium are shown in Table II.

Table II

Determination of vanadium in the presence of various ions

Sample No.	Vanadium present, mg	Vanadium found, mg	Ion present	Remarks
1	0·106	0·106	100 mg of Fe	
2	0·245	0·247	100 mg of Cr	
3	0·245	0·247	100 mg of Al	
4	0·123	0·118	100 mg of Cu	
5	0·123	0·124	30 mg of Ti	30 mg of Ti alone gave a pale yellow chloroform extract (optical density, −0·015)
6	0·123	0·114	50 mg of W, as tungstate	Several extractions necessary to remove complex from hydrated tungsten oxide precipitated
7	0·123	0·148	15 mg of $K_2Cr_2O_7$	Dichromate oxidises reagent to give a yellow colour and thus high results for vanadium
8	0·213	0·200	50 mg of $K_2Cr_2O_7$	Dichromate reduced with ferrous sulphate; vanadium re-oxidised by boiling with nitric acid
9	0·213	0·192	50 mg of $K_2Cr_2O_7$	Dichromate reduced with ferrous sulphate, vanadium re-oxidised with permanganate, and excess of permanganate destroyed with sodium nitrite
10	0·213	0·205	50 mg of $K_2Cr_2O_7$	As for sample No. 9, but urea added to destroy the excess of nitrite
11	0·213	0·212	50 mg of $K_2Cr_2O_7$	As for sample No. 9, but the drop or two of permanganate added in excess destroyed by hydrochloric acid
12	0·108	0·108	250 mg of Fe_2O_3 + 250 mg of Cr_2O_3	Sample fused with 6 g of sodium carbonate and 4 g of sodium tetraborate in a platinum crucible, dissolved in 50 ml of sulphuric acid (1 + 4), diluted to 100 ml, and a 25-ml aliquot treated as for sample No. 11
13	0·054	0·056	250 mg of Fe_2O_3 + 250 mg of Cr_2O_3	As for sample No. 12

These results show that vanadium can be readily determined in the presence of commonly associated ions. Iron and chromium, often interferences in determining vanadium, pose no problems; when chromium is present as dichromate, a method for reducing it to the tervalent state and of re-oxidising the vanadium without oxidising the chromium must be used. Reduction with ferrous iron and re-oxidation with permanganate is rapid and satisfactory. As the procedure requires a solution 5 to 9 *M* in hydrochloric acid, the drop or two of permanganate added in excess is readily destroyed by the acid and causes no interference; the amount of

chlorine thus produced is negligible. The use of nitrite to remove permanganate results in some reduction of the vanadium; this effect is lessened when urea is added to destroy the excess of nitrite.

Oxidation of a sulphuric acid solution with nitric acid, after treatment with ferrous iron to reduce dichromate, is adequate provided the aliquot ultimately taken for analysis is not more than 5 per cent. in concentrated nitric acid and any nitrites present are destroyed with urea. When 50 mg of molybdenum are present, as ammonium molybdate, only part of the vanadium is extracted on one treatment with benzoylphenylhydroxylamine in chloroform; continued extraction with the reagent gives further purple extracts, but there is some hold-up of the vanadium and, after four extractions, 90 per cent. of the vanadium is recovered. No difficulties are encountered, however, with the amounts of molybdenum associated with vanadium in steels. Similarly, zinc, cobalt, nickel, copper, magnesium, calcium, manganese, titanium, zirconium, arsenic, tin, tungsten, silicon, phosphorus, carbon and sulphur present in steels, chromites or chrome-magnesite refractories do not interfere.

Determination of Vanadium in Steels and Chrome Ores

Steels—

Dissolve a sample of appropriate size (containing approximately 1 mg of vanadium) in 75 ml of dilute sulphuric acid (1 + 4), and add a few drops of nitric acid to oxidise iron and tungsten. Filter through a sintered-glass crucible, wash with hot water, and dilute to 100 ml. Take a 25-ml aliquot, add potassium permanganate (approximately 0·1 *N*) dropwise until a faint pink colour persists, make the solution 5 to 9 *M* in hydrochloric acid, and extract with benzoylphenylhydroxylamine reagent solution. Dilute to 50 ml with chloroform, and measure the optical density at 530 mμ.

Results—This procedure was applied with excellent results to a high-speed and to a chrome-vanadium steel; 1 hour is sufficient to determine the vanadium content of steels by this method. The results obtained were—

Sample	Vanadium present, %	Vanadium found, %
B.C.S. steel 241/1	1·57	1·55
B.C.S. steel 244	0·24	0·25

Chromite and chrome-magnesite refractory—

Fuse 0·5 g of the sample with a mixture of sodium carbonate and sodium tetraborate in a platinum crucible. Set aside to cool, and dissolve the melt by warming in 50 ml of dilute sulphuric acid (1 + 4); dilute accurately to 100 ml in a calibrated flask. Take a 25-ml aliquot, and reduce dichromate to chromic ion by adding a solution of ferrous sulphate until a pure green colour is obtained. Add potassium permanganate (approximately 0·1 *N*) until a faint pink colour persists, make the solution 5 to 9 *M* in hydrochloric acid, and extract with benzoylphenylhydroxylamine reagent solution; complete the determination as described on p. 571.

Results—Results obtained by this procedure on oxide mixtures are shown in Table II; satisfactory results were obtained on available ores containing from 0·03 to 0·11 per cent. of vanadium. An ore sample to which 0·213 mg of vanadium had been added gave a recovery of 0·214 mg after allowance for the vanadium content found in the ore alone.

Fusion with sodium peroxide in a nickel crucible was also used for these samples without difficulty. In this method the resulting melt was extracted with water, and the solution heated to boiling for 10 minutes; when the solution had cooled, 5 g of ammonium carbonate were added, the solution was filtered, and the precipitate washed several times with hot water. The filtrate was neutralised with cold diluted sulphuric acid (1 + 1), a known aliquot taken and treated as described above.

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An Improved Colorimetric Method for Determining Ferrocyanide Ion, and its Application to Molasses

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An accurate and reproducible colorimetric method for determining 5 to 80 μg of ferrocyanide ion is described. The procedure can be used for determining residual ferrocyanide in beet molasses, and the presence of up to 3 ml of diluted molasses ($\frac{1}{4}$ strength) has no adverse effect. The recovery of ferrocyanide added to molasses averaged 100·3 $\pm$ 3·2 per cent. and was not significantly affected by fermentation treatment.

In the production of citric acid by fermentation of molasses, ferrocyanide is used as precipitant.[1 to 5] Since it has been shown that the concentration of residual ferrocyanide affects the yield of citric acid,[4,5] accurate control and hence accurate measurement of ferrocyanide ion in beet molasses is essential in such fermentation processes.

The colour reaction between ferrocyanide and ferric ions in aqueous solution has been previously used for determining 30 to 120 μg of residual ferrocyanide in molasses[5] and in indirect quantitative methods for determining sugar in blood[6,7] and reducing substances in milk powders.[8,9] These methods, however, have disadvantages that preclude the attainment of accurate reproducible results in the determination of small amounts of ferrocyanide: (*a*) sensitivity is too low[5] to permit accurate measurement of amounts of ferrocyanide below 50 μg, (*b*) the gum ghatti used as protective colloid[7,8] does not always prevent precipitation of ferric ferrocyanide after prolonged standing[9] and (*c*) colour formation does not obey Beer's law[8] unless readings are made within 1 minute.[9]

In the modified method described in this paper, these disadvantages have been overcome.

Development of the method

A study of the absorption characteristics of the colour and the effects of the concentrations of the various components was made. Although Chapman and McFarlane[8] recommended that the optical densities of sample solutions should be measured at 660 mμ and Martin[5] used 675 mμ, our measurements (with a Coleman Junior spectrophotometer) indicated that maximum absorption for the ferric ferrocyanide complex was between 690 and 700 mμ. The 690-mμ filter of an Evelyn colorimeter was therefore used throughout this work. The optimum range for accurate measurement of the ferric ferrocyanide colour (giving between 85 and 15 per cent. absorption with an Evelyn colorimeter) was from 5 to 80 μg of ferrocyanide ion per 8 ml of final solution; concentrations above about 200 μg of ferrocyanide ion per 8 ml caused precipitation of the blue complex within 1 hour.

Concentrations of reagents—

The concentration of ferric ion necessary for maximum colour intensity was not critical at the higher levels of ferrocyanide, but became increasingly so as the level of ferrocyanide

was decreased (see Fig. 1). The optimum concentration of ferric ion at low levels of ferrocyanide was 6 mg per 8 ml (0·075 per cent.); the levels suggested by Chapman and McFarlane,[8] Folin[6] and Martin[5] were 0·009, 0·058 and 0·833 per cent., respectively.

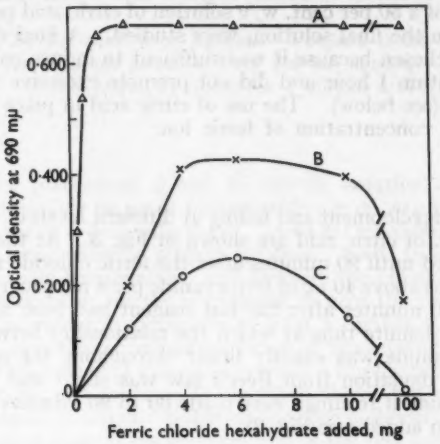

Fig. 1. Effect of concentration of ferric chloride on colour intensity in presence of 0·0375 *N* hydrochloric acid: curve A, 60 μg of ferrocyanide ion; curve B, 40 μg of ferrocyanide ion; curve C, 30 μg of ferrocyanide ion. Final volume of solution 8 ml; optical densities measured after 1 hour

In previous methods, the acid medium necessary for the formation of ferric ferrocyanide was provided by phosphoric,[6] trichloroacetic[8] or citric[5] acid. We compared the effects of hydrochloric and citric acids. At the optimum concentration of hydrochloric acid (0·025 to 0·050 *N* in the final solution, including the acid introduced with the ferric chloride reagent),

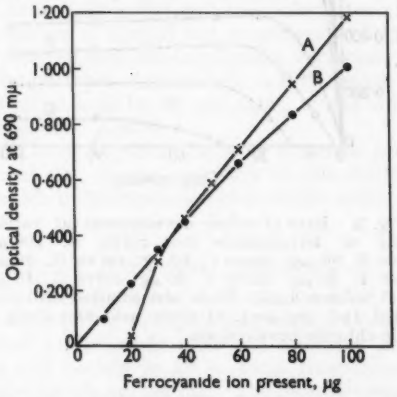

Fig. 2. Effect of type of acid on sensitivity in presence of 6 mg of ferric chloride hexahydrate: curve A, 0·0375 *N* hydrochloric acid; curve B, 12·5 per cent. of citric acid. Final volume of solution 8 ml; optical densities measured after 90 minutes

the relationship between concentration of ferrocyanide and optical density was linear only between 40 and 100 μg of ferrocyanide per 8 ml of final solution; the optical density decreased sharply at levels below 40 μg of ferrocyanide (see Fig. 2).

With citric acid, the rate of colour formation and the final colour intensity were increased at low concentrations of ferrocyanide. A deep yellow colour (green when mixed with the blue) resulted from the formation of ferric citrate,[10] but, as this colour did not absorb in the 690-mμ region, it did not interfere with the determination of ferrocyanide. The effects of between 0·5 and 3·5 ml of a 50 per cent. w/v solution of citric acid per 8 ml, *i.e.*, from 3 to 22 per cent. of citric acid in the final solution, were studied. A final concentration of 12·5 per cent. of citric acid was chosen because it was sufficient to induce colour development at low levels of ferrocyanide within 1 hour and did not promote excessive fading at higher concentrations of ferrocyanide (see below). The use of citric acid in place of hydrochloric acid did not affect the optimum concentration of ferric ion.

STABILITY OF COLOUR—

The rates of colour development and fading at different levels of ferrocyanide in solutions containing 12·5 per cent. of citric acid are shown in Fig. 3. At the 10-μg level, full colour intensity was not attained until 90 minutes after the ferric chloride reagent had been added, whereas, at concentrations above 40 μg of ferrocyanide per 8 ml, progressive fading (5 per cent. per hour) began 20 to 30 minutes after the last reagent had been added. It was therefore impossible to select any definite time at which the relationship between optical density and concentration of ferrocyanide was exactly linear throughout the entire range covered by the method. However, deviation from Beer's law was slight and occurred only at levels above 40 μg of ferrocyanide if readings were made 60 to 90 minutes after the ferric chloride reagent solution had been added (see Fig. 2).

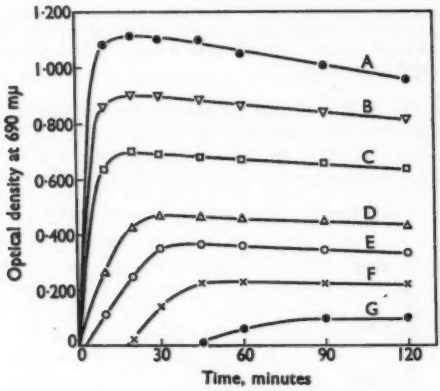

Fig. 3. Rate of colour development at various levels of ferrocyanide ion: curve A, 100 μg; curve B, 80 μg; curve C, 60 μg; curve D, 40 μg; curve E, 30 μg; curve F, 20 μg; curve G, 10 μg. Final volume 8 ml. Each test solution also contained 12·5 per cent. of citric acid and 6 mg of ferric chloride hexahydrate.

An attempt was made to promote additional colour by heating samples of standard ferrocyanide solution during the colour-development period. Heating for 1 hour at 60° C, as previously recommended,[5] appeared to have no significant effect on the rate of colour formation. Nevertheless, with a sucrose-rich substrate, such as molasses, it is thought that heating could be a potential source of error, owing to the possibility of caramelisation and the subsequent increased colour of the sample. Such a colour would be difficult to correct for, as it would probably not develop at the same rate in the "molasses colour" blank as in the test solution in presence of ferric ion.

Method

Reagents—

Ferric chloride reagent solution, 0·6 per cent. w/v, in 0·1 N hydrochloric acid—Prepare from ferric chloride hexahydrate. This solution should be stored in a dark bottle and freshly prepared every 2 weeks.

Citric acid solution, 50 per cent. w/v, aqueous—Prepare from citric acid monohydrate.

Standard potassium ferrocyanide solution, 1 mg per ml, aqueous—Prepare from potassium ferrocyanide trihydrate. This solution is stable for 1 month and should be stored in a dark bottle.[11]

Procedure—

Transfer an aliquot (maximum 5 ml) of sample solution, diluted standard ferrocyanide solution containing 5 to 80 μg of ferrocyanide ion or water (blank) to a colorimeter tube, and adjust its volume to 5·0 ml with water. Add 2·0 ml of 50 per cent. citric acid solution and then 1·0 ml of ferric chloride reagent solution. Swirl to mix thoroughly, set aside for 60 to 90 minutes at room temperature, and measure the optical density at 690 mμ with an Evelyn or similar colorimeter; set the instrument to give 100 per cent. transmission with the blank solution. (The colour is stable for at least a further 60 minutes, and deviation from Beer's law is slight over the recommended range of ferrocyanide concentrations.)

To correct for the dark colour of molasses, transfer a duplicate aliquot of sample solution to another colorimeter tube, and continue as described above, but substitute 1·0 ml of water for the ferric chloride reagent solution. Subtract the optical density of this sample, measured against a normally prepared reagent blank, from that of the test solution.

Application of the method to molasses

In applying the proposed method to molasses, the possible effects of individual components, the colour of the molasses, the combined effect of all components and the possible effect of the fermentation treatment were studied. Throughout this work, beet molasses were diluted to $\frac{1}{4}$ strength; such solutions contained 12 per cent. w/v of total sugar, *i.e.*, approximately the same concentration used in previous studies of fermentation.[3,4]

Sucrose, the predominant carbohydrate component of molasses, was found to have no effect on the method, even when as much as 1250 mg (equivalent to 5 ml of a 25 per cent. solution) were added per test.

The concentration of citric acid in the molasses substrates varies between 0 and 10 per cent.,[4] but, as the determination is carried out in presence of 12·5 per cent. of citric acid, aliquots of such samples would not introduce significant variation. For example, the addition of 3·0 ml of molasses containing 10 per cent. of citric acid to 8 ml of test solution would increase the total concentration of citric acid by 30 per cent., which would have no adverse effect (see "Concentrations of Reagents," p. 574).

Before the effect of different concentrations of molasses could be studied or recovery experiments could be carried out, it was necessary to remove traces of metals that would cause loss of added ferrocyanide by precipitation of insoluble salts. Two methods of treatment were used.

Treatment with ferrocyanide and ferric chloride—

The sample of molasses was sterilised by heating at 120° C for 45 minutes, and interfering cations were then removed by precipitation with potassium ferrocyanide (0·06 g per 100 ml of molasses),[4] added immediately after the molasses had been removed from the steriliser. After filtration and cooling in air to room temperature, the molasses contained about 10 μg of residual ferrocyanide ion per ml. The molasses was acidified by adding 1 ml of concentrated hydrochloric acid to each 99 ml of molasses and was then divided into five portions. The residual ferrocyanide was precipitated by adding amounts of ferric chloride hexahydrate to each portion so as to obtain final concentrations of 0·60, 0·30, 0·20, 0·15, and 0·12 per cent. in the solutions of molasses. Each of these concentrations was sufficient to precipitate the residual ferrocyanide[5] without significantly decreasing the concentration of ferric ion present. After centrifugation, 1, 2, 3, 4, and 5-ml portions, respectively, of the five solutions of molasses were used for the tests. (These portions introduced the 6 mg of ferric chloride required for the determination.) Determinations were carried out at levels between

0 and 80 μg of ferrocyanide ion, with 2-ml portions of 50 per cent. citric acid and a final volume of 8 ml being used per test. "Molasses colour" blanks were included for each level of molasses present.

TABLE I

EFFECT OF MOLASSES ON OPTICAL DENSITY AT DIFFERENT LEVELS OF FERROCYANIDE

Samples of molasses were diluted (¼ strength), treated with potassium ferrocyanide to remove interfering metals and then treated with ferric chloride to remove residual ferrocyanide. The final volume of solution used in each test was 8 ml

Amount of ferrocyanide ion present, μg	Optical density in presence of— 1 ml of treated molasses	2 ml of treated molasses	3 ml of treated molasses	4 ml of treated molasses	5 ml of treated molasses
5	0·049	0·046	0·048	0·039	0·036
10	0·096	0·097	0·095	0·086	0·079
15	0·145	0·136	0·141	0·132	0·131
20	0·194	0·187	0·197	0·199	0·164
40	0·385	0·382	0·414	0·379	0·357
60	0·559	0·560	0·550	0·542	0·537
80	0·750	0·754	0·750	0·706	0·699

The dark brown colour of ferrocyanide-treated molasses, when corrected for by using a blank solution, did not affect the sensitivity of the method until more than 3 ml had been added (see Table I); the addition of 4- and 5-ml portions resulted in decreased sensitivity and some erratic readings. The formation of ferric ferrocyanide in beet molasses was in satisfactory accord with Beer's law for amounts of ferrocyanide ion between 5 and 80 μg, but there was a significant decrease in sensitivity when more than 80 μg of ferrocyanide were present.

Samples of molasses treated as described above contained all the original colouring matter and were therefore suitable for studying the effect of different concentrations of molasses. However, owing to the presence of ferric chloride, such samples were not suitable for recovering added ferrocyanide because of the danger of precipitation before the determination. A second treatment for the preliminary separation of interfering heavy metals was therefore used.

TREATMENT WITH CATION-EXCHANGE RESIN—

A 300-g amount of a cation-exchange resin (Amberlite IR-120) was added to 1000 ml of molasses, the mixture was vigorously shaken for 2 hours at 35° C, and the resin was removed by centrifugation. This treatment was repeated four times, fresh resin being used each time. Samples of molasses so prepared were used for recovery experiments; these samples were much lighter in colour than the original and gave no precipitate with added ferrocyanide. Amounts of ferrocyanide between 5 and 80 μg per ml were added to portions of these samples,

TABLE II

RECOVERY OF FERROCYANIDE ADDED TO RESIN-TREATED MOLASSES

Samples of molasses were diluted (¼ strength) and then treated with Amberlite IR-120 to remove interfering cations

Amount of ferrocyanide ion added per ml of treated molasses, μg	Amount of ferrocyanide found per ml of treated molasses on— first day, μg	second day, μg	third day, μg	Average amount found, μg	Recovery, %
5·0	5·0	5·1	4·6	4·9	98·0 ± 5·0
10·0	10·0	10·6	9·5	10·0	100·0 ± 5·5
20·0	20·2	21·0	19·6	20·3	101·5 ± 3·5
40·0	40·0	40·5	40·4	40·3	100·8 ± 0·6
60·0	59·9	61·7	58·6	60·1	100·2 ± 2·6
80·0	81·2	79·2	82·0	80·8	101·0 ± 1·8
				Mean	100·3 ± 3·2

as a 0·20 per cent. w/v stock solution of potassium ferrocyanide trihydrate in de-mineralised molasses. The amounts of ferrocyanide present in 1-ml portions of these solutions were determined on three successive days.

The recoveries of added ferrocyanide ion from resin-treated beet molasses are shown in Table II. The relatively low variability of the results, with average recoveries close to 100 per cent., indicates the accuracy attainable.

EFFECT OF FERMENTATION—

The possible effect of the fermentation process *per se* was also studied. Additions of 10 and 20 μg of ferrocyanide per ml were made to portions of molasses that had undergone submerged fermentation[3,4] for approximately 2 days. The results are shown in Table III and indicate that the method was not significantly affected.

TABLE III

RECOVERY OF FERROCYANIDE ADDED TO FERMENTED MOLASSES

Initial ferrocyanide content, μg per ml	With 10 μg of ferrocyanide added per ml		With 20 μg of ferrocyanide added per ml	
	Ferrocyanide content found, μg per ml	Recovery, %	Ferrocyanide content found, μg per ml	Recovery, %
54·2	64·0	98·0	73·6	97·0
46·8	56·4	96·0	66·6	99·0
37·0	47·5	105·0	58·4	107·0
16·0	26·5	105·0	—	—
4·5	14·7	102·0	25·1	103·0
	Mean	101·2	Mean	101·5

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Paper Chromatography of some Organo-tin Compounds

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The paper partition chromatography of some organo-tin compounds, R_nSnX_{4-n}, is described. The developing solvents used are butanol - pyridine - water, butanol - ethanol - water, butanol - ammonia - water and aqueous pyridine. The organo-tin compounds are detected by spraying with catechol violet after initial oxidation of compounds of the types R_4Sn and R_3SnX by ultra-violet irradiation, which renders them sensitive to the spray. Diphenyltin and triphenyltin compounds can be separated and recovered quantitatively.

ORGANO-TIN compounds are those in which a tin atom is directly linked to at least one carbon atom. Such derivatives of tin^{IV} can be divided into four groups, *viz*, R_4Sn, R_3SnX, R_2SnX_2 and $RSnX_3$, where R is an alkyl or aryl group and X is acid. Of these four groups the mono-alkyl or -aryl compounds, $RSnX_3$, are not of any special interest, and the only important use of the tetra-compounds, R_4Sn, so far is as additives to chlorinated hydrocarbons in

transformer oils. On the other hand, certain dialkyltin compounds (R_2SnX_2) are widely used as stabilisers for poly(vinyl chloride), and members of the group R_3SnX, because of their powerful biocidal properties, are likely to become of increasing importance as fungicides and bactericides.

Detection and determination of small amounts of members of the two last-named groups are thus of interest, and details of several approaches to the problem have been published. Small amounts of tri- and dialkyltin compounds can be separated by an extraction technique and subsequent photometric determination of the compounds as such by means of dithizone.[1] Mixtures of di- and triphenyltin compounds with inorganic tin salts can be separated by extraction in the presence of tartrate and then extraction of the pyrrolidine dithiocarbamate complexes. The separated compounds are then wet-ashed and tin is determined polarographically.[2] This procedure has been applied to the determination of small amounts of triphenyltin compounds on plant materials.[3] A few reports have appeared on the polarography of di- and trialkyltin compounds, but so far this method has not been particularly successful.[4] The paper chromatography of some organo-tin compounds has recently been described.[5] From the R_F values reported it is evident that the separation of tetraphenyltin from both the di- and triphenyltin compounds is possible by this method, and it was hoped that, by suitable choice of solvent, further separations could be achieved.

Experimental

Organo-tin compounds—

Commercial samples of the compounds listed below were obtained.
Tetraphenyltin.
Triphenyltin compounds.
Tributyltin laurate and abietate.
Dibutyltin dilaurate and dichloride.
Triethyltin abietate.

The other compounds used were prepared at the Organic Chemistry Institute T.N.O., Utrecht, The Netherlands.

Purification—

The dibutyltin dichloride was recrystallised from light petroleum, the phenyltin compounds from chloroform and the tributyltin chloride was redistilled *in vacuo*.

Developing solvents—

(*a*) *Pyridine*—A 60 per cent. v/v solution of analytical-reagent grade pyridine in water.
(*b*) *Butanol - pyridine - water* (7·5 + 3·5 *and saturated with water*).
(*c*) *Butanol - ethanol - water* (3 + 1 *and saturated with water*).
(*d*) *Butanol - ammonia - water* (N *in ammonia and saturated with water*).

Chromatographic paper—

Whatman No. 1 *paper "for chromatography"*—In reels 6 inches wide.

Spray reagent—

Catechol violet solution, 0·1 per cent. in 95 per cent. ethanol—This material does not react with tetra-alkyl or tetra-aryltin compounds. It gives a reddish mauve colour with tri-compounds, but is not particularly sensitive to them. Di- or mono-compounds, however, show the blue colour given by inorganic tin compounds with catechol violet, and this reaction is adequately sensitive. It was found that irradiation of chromatograms of tetra- or tri-compounds for about 10 minutes with an ultra-violet lamp caused sufficient oxidation to di- and mono-compounds for them to be readily detected by the reagent. The sprayed chromatograms were found to be stable for a considerable time, the yellow background fading to grey and the blue spots losing a little of their intensity.

Procedure—

Qualitative—The paper was cut to allow up to 20 inches development, and solutions were applied at 1-inch intervals by means of a capillary; side margins of 1½ inches were allowed. Descending-solvent chromatograms were developed in the dark at 24° ± 1° C for about 16 hours (usually overnight). In this time the solvent front moved about 12 to 16 inches from the line of application. The chromatograms were allowed to dry and were then

irradiated for about 10 minutes with an ultra-violet lamp; they were then sprayed with the catechol violet solution. R_F values were measured to the centres of the blue spots, but when tailing occurred measurement was made to the front of the spot.

Quantitative—Measured volumes (about 0·25 ml) of solutions of known concentrations were applied across the width of the paper by means of a micrometer syringe; side margins of 1 inch were allowed. After development, irradiation and spraying, as described above, the blue bands obtained were cut out with ½-inch margins on both sides and wet-ashed with sulphuric and nitric acids. The inorganic tin in the resulting solutions was determined turbidimetrically with 4-hydroxy-3-nitrophenylarsonic acid.[6]

The total tin content of the chromatographed mixture was checked by wet-ashing the same volume of solution as applied to the paper.

Results

Table I shows R_F values found for several organo-tin compounds by the qualitative procedure. Table II shows recoveries of a diphenyltin and a triphenyltin compound by the qualitative procedure.

Discussion of results

Table I shows that the acid radicle X in R_nSnX_{4-n} does not affect the R_F value, and this indicates hydrolysis of the compounds by the basic solvents to the corresponding hydroxides. Detection of pyridinium chloride when pyridine was present in the developing solvent tends to confirm this.

Of these hydroxides, those of trialkyl- and triaryltin are stable, but those of the di-compounds tend to lose water to form polymeric oxides $(R_2SnO)_n$. These latter are insoluble

TABLE I

R_F VALUES

Compound	Developing solvent: Pyridine, 60%	Butanol - pyridine - water	Butanol - ammonia - water	Butanol - ethanol - water
Me_2SnCl_2	0·36‡	0·55†	0·03	0·67†
Me_3SnCl	0·35	0·25	—	0·32
$EtSnCl_3$	Streaks to 0·8	Streaks length of paper	Streaks length of paper	Streaks length of paper
Et_2SnCl_2	0·36‡	0·80†	0·16‡	0·98†
Et_2SnOAc	0·40	0·85†	—	0·95†
Et_3SnOH	0·88	0·94	0·94	0·95
Pr_2SnCl_2	0·00†	0·9†	Streaks to 0·52	0·98
$(Pr_2Sn)_2O$	0·85	0·94	—	—
$(iso\text{-}Pr_2Sn)_2O$	0·87	0·94	—	—
$Pr_2SnCH_2CH_2COONa$	0·82	0·90	0·83	—
$BuSnCl_3$	Streaks to 0·8	0·99†	Streaks length of paper	Streaks length of paper
Bu_2SnCl_2	0·00*	0·93	0·00†	0·98*
Bu_2Sn dilaurate	0·00*	0·95	—	0·96
Bu_2SnCl	0·85	0·92	0·95	0·94
Bu_2Sn laurate	0·82	0·92	—	0·94
$(Bu_2Sn)_2O$	0·82	0·97	—	0·96
Bu_2Sn abietate	0·84	0·97	0·94	—
Hexabutyl distannane	—	0·94 (0·8, 0·3§)	0·95 (0·8§, 0·3§)	—
Bu_4Sn	0·00	0·93	0·94	0·92
n-$Hexyl_2Sn$ dilaurate	0·00	0·95	—	0·95
n-$Octyl_2Sn$ dilaurate	0·00	—	—	0·94
n-$Octyl_2SnCl$	0·00	0·94	0·93	0·95
n-$Octyl_4Sn$	0·00	0·90	0·94	0·83
$PhSnCl_3$	Streaks to 0·85	0·99†	Streaks length of paper	Streaks length of paper
Ph_2SnCl_2	0·00	0·00	0·00	0·95†
Ph_3SnCl	0·85	0·95	0·97	0·97
Ph_3SnOAc	0·85	0·95	0·97	0·97
Ph_4Sn	0·00	0·00	0·00	0·00
$SnCl_4$	0·00	0·00	0·00	0·00*

* Slight tail. † Severe tailing. ‡ Elongated spot. § Probably impurity.

in most solvents and would lead to zero R_F values, as was found in 60 per cent. pyridine from the dibutyl compounds upwards. Because of the stability of the tri-compounds and their general insolubility in water, coupled with their solubility in organic solvents, high R_F values may be expected.

The only exception to this occurs with the trimethyl compound; this may possibly be due to appreciable solubility in water.

Quantitative measurements show satisfactory separation and recovery of di- and triphenyltin compounds within certain concentration limits. In Table II low recoveries are shown for amounts of diphenyltin dichloride above 1 mg, and this may have been due to overloading of the paper at these levels.

TABLE II

RECOVERY OF ORGANO-TIN COMPOUNDS

Ph_2SnCl_2 applied, mg	Ph_3SnCl applied, mg	Ph_2SnCl_2 found, mg	Ph_3SnCl found, mg	Recovery of Ph_2SnCl_2, %	Recovery of Ph_3SnCl, %
With 60 per cent. pyridine as developing solvent—					
—	0·39	—	0·372	—	96
—	0·39	—	0·39	—	100
0·455	0·52	0·45	0·52	99	100
0·26	0·120	0·25	0·119	96	99
0·635	0·355	0·63	0·352	99	99·5
1·58	0·097	1·43	0·057	91	58
0·76	—	0·76	—	100	—
3·90	—	3·05	—	78	—
With butanol - pyridine - water as developing solvent—					
—	0·39	—	0·375	—	96·5
—	0·39	—	0·38	—	97
0·455	0·52	0·425	0·55	93	106
—	0·120	—	0·125	—	105
0·635	0·355	0·60	0·365	94	103
1·58	0·097	1·43	0·097	91	100
0·76	—	0·755	—	99	—
3·90	—	3·52	—	90	—

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Identification of Barbiturates from their Infra-red Spectra

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A standardised technique has been developed for preparing potassium bromide discs of barbituric acids for infra-red analysis, and the spectra obtained do not depend on the methods used for purifying and preparing the samples. The technique is suitable for identifying toxicological samples, as only small amounts are needed.

BECAUSE of the importance of barbiturate drugs in medical and toxicological work, numerous physical methods of identification have been examined.[1] Infra-red absorption and X-ray diffraction techniques have been the most promising, as both methods give many possible points of identification, varying in relative intensities, by which a compound can be distinguished. Both methods suffer from the disadvantages that they are sensitive to barbiturate

polymorphism.[2] This has been overcome in X-ray work by a standardised technique of crystallisation,[3] and a standardised procedure for the alternative infra-red method is described in this paper.

The use of infra-red spectra for identifying barbiturates was first demonstrated for chloroform solutions of barbiturates isolated from toxicological specimens.[4] About 5 mg of sample were required and there was the further disadvantage that the solution spectra of some barbiturates are rather similar. Solid-state spectra of barbiturates have been recorded,[5,6,7] but they show the effects of polymorphism on the infra-red spectrum of some samples and of polymorphic changes induced by grinding during preparation of the sample.[8] The copper - pyridine[6] and *p*-nitrobenzyl[5] complexes with barbiturates do not appear to exhibit polymorphism, and their spectra have been recorded.

The proposed procedure is simple and requires less than 1 mg of solid barbiturate. For each of twenty barbiturates, the spectrum obtained is independent of the initial state of

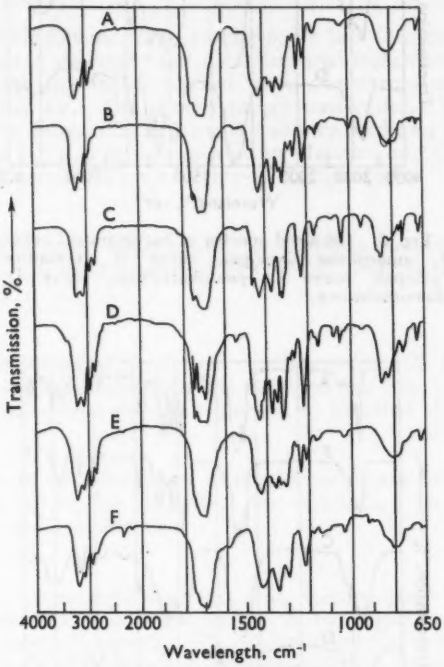

Fig. 1. Infra-red spectra of barbiturates: curve A, amobarbitone; curve B, aprobarbitone; curve C, barbitone; curve D, butabarbitone; curve E, Butethal; curve F, cyclobarbitone

the sample (whether amorphous or crystalline in any of a number of polymorphic forms) and also of spectral changes brought about during preparation of a potassium bromide disc.

Experimental

Spectra were recorded between 4000 and 650 cm^{-1} with a Perkin-Elmer model 21 double-beam spectrophotometer fitted with a sodium chloride prism. The barbiturate to be identified was prepared as a potassium bromide disc after about 0·7 to 0·8 mg had been ground by hand for 5 minutes with about 300 mg of potassium bromide (infra-red quality, obtained from the Harshaw Chemical Co., Cleveland, Ohio, U.S.A.). The melting-point of the sample was determined by using a Kofler block, and the disc was placed in an oven maintained at a temperature 10° C above the melting-point. After being heated for 30 minutes, the disc was removed and rapidly cooled to room temperature, and the spectrum was recorded.

The barbituric acids used were obtained from commercial drug firms and purified by recrystallisation to constant melting-point, usually from aqueous ethanol; when necessary, other crystalline modifications were also prepared.[2] To check each barbiturate standard before it was incorporated in a disc, paraffin mulls were prepared, with the minimum grinding necessary to produce a sharp spectrum.

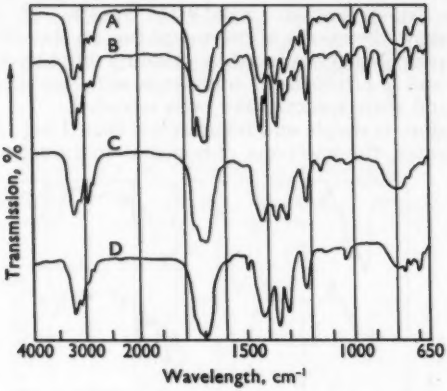

Fig. 2. Infra-red spectra of barbiturates: curve A, amorphous Cyclopal; curve B, crystalline Cyclopal; curve C, pentobarbitone; curve D, phenobarbitone

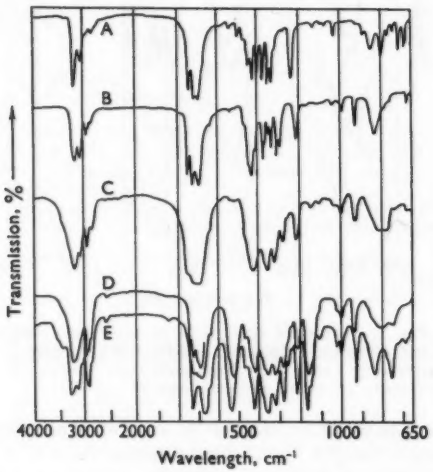

Fig. 3. Infra-red spectra of barbiturates: curve A, Rutonal; curve B, Sandoptal; curve C, secobarbitone; curve D, Thiamylal (initial spectrum); curve E, Thiamylal (spectrum after storage of disc)

Results

Clear discs sometimes became cloudy when heated, but, except for an increase in the scattered light at short wavelengths, the spectra were unimpaired. The barbiturates studied are listed below, and, when the infra-red spectrum obtained by using this method cannot be found in the literature, it is shown in Fig. 1, 2 or 3.

AMOBARBITONE—

The spectrum of the amorphous amobarbitone (5-methyl-5-*iso*amylbarbituric acid, melting-point 159° C) obtained after treatment of discs is shown in Fig. 1. The spectrum of a disc left for 19 days was the same as those reported by Chatten and Levi[5] and Manning and O'Brien,[7] showing that the samples slowly crystallised within the discs to the crystalline form I.

APROBARBITONE—

The amorphous aprobarbitone (5-allyl-5-*iso*propylbarbituric acid, melting-point 144° C) resulting from treatment of the discs changed fairly rapidly to a crystalline form, so that the spectra of duplicate discs, as shown in Fig. 1, are not identical. Two days after the discs had been treated, their spectra had changed completely to the crystalline form III.[2]

BARBITONE—

Barbitone (5:5-diethylbarbituric acid, melting-point 191° C) exists in several crystalline modifications having different spectra,[2,8] but discs prepared from these different forms, after being heated at 200° C and then rapidly cooled, gave a spectrum (see Fig. 1) indicating a mixture of forms I and II or IV. The relative proportions varied a little between duplicate discs, and 4 weeks later the proportion of form II had increased and the spectrum indicated the same mixture of forms I and II as was reported by Manning and O'Brien.[7] This must be a stable mixture, as the spectrum did not appear to change again.

BUTABARBITONE—

The spectrum of crystalline butabarbital (5-ethyl-5-(α-methyl)propylbarbituric acid, melting-point 86° C) obtained after treatment of the disc is shown in Fig. 1, as it has not been previously reported.

BUTETHAL—

Butethal (5-ethyl-5-*n*-butylbarbituric acid, melting-point 127° C) is polymorphic, but the smooth spectrum of a barbiturate glass was obtained (see Fig. 1) after treatment of the disc. The barbiturate crystallised slowly in the disc to butethal II.[5,6]

CYCLOBARBITONE—

An amorphous form of cyclobarbitone (5-ethyl-5-*cyclo*hexenylbarbituric acid, melting-point 172° C), which did not readily crystallise, was obtained. The spectrum had unusual peaks at 2330 and 2155 cm^{-1} (see Fig. 1).

CYCLOPAL—

Cyclopal (5-allyl-5-*cyclo*pentenylbarbituric acid, melting-point 140° C) existed in the disc in the amorphous form after treatment (see Fig. 2), but began to show evidence of change to the crystalline form soon afterwards. The spectrum of the crystalline form (a sample obtained from the Upjohn Co., Kalamazoo, Michigan, U.S.A.) is shown in Fig. 2, as it is somewhat different from the spectrum reported by Chatten and Levi.[5]

DIAL—

The spectra of the treated discs of Dial (5:5-diallylbarbituric acid, melting-point 174° C) were the same as others reported in the literature.[6,7] There was slight sharpening of the spectra of discs when they were recorded 1 month later.

HEXOBARBITONE—

With only a slight loss in sharpness, the spectrum of hexobarbitone (1:5-dimethyl-5-*cyclo*hexenylbarbituric acid, melting-point 146° C) was the same as those reported by Chatten and Levi[5] and Manning and O'Brien.[7] The spectrum was unusually sharp after the disc had been kept for 1 month.

KEMITHAL—

Discs prepared from different crystalline forms of Kemithal (5-allyl-5-*cyclo*hexenylthiobarbituric acid, melting-point 125° C) gave form III[2] after treatment. This was unchanged after 5 weeks.

MEPHOBARBITONE—

The spectrum obtained for mephobarbitone (1-methyl-5-ethyl-5-phenylbarbituric acid, melting-point 181° C) was less sharp than those previously reported,[6,7] but sharpened after the disc had been kept.

NOSTAL—

Discs of both crystalline forms of Nostal (5-*iso*propyl-5-(β-bromo)allylbarbituric acid, melting-point 184° C) gave the spectrum of form I[2] after treatment. The spectrum sharpened slightly after the discs had been kept.

PENTOBARBITONE—

The spectrum of the amorphous form of pentobarbitone (5-ethyl-5-(α-methyl)butylbarbituric acid, melting-point 132° C) obtained after heating the disc is shown in Fig. 2. This changed after a few weeks to that of the crystalline form I, II or III,[2,5,6] but was much sharper than any of the reference spectra and had peaks in several places where only inflexions appear in other solid-state spectra.

PHENOBARBITONE—

The spectrum obtained for phenobarbitone (5-ethyl-5-phenylbarbituric acid, melting-point 178° C) was that of the amorphous form (see Fig. 2). There was an initial sharpening of the spectrum after the disc had been kept, but this did not increase after 2 months and does not appear to indicate the presence of any known crystalline form.

PROBARBITONE—

The spectrum of the disc containing probarbitone (5-ethyl-5-*iso*propylbarbituric acid, melting-point 206° C) after treatment was the normal spectrum of crystalline probarbitone.[5,6] There was slight sharpening after the disc had been kept for 4 weeks.

RUTONAL—

The spectrum of crystalline Rutonal (5-methyl-5-phenylbarbituric acid, melting-point 229° C, obtained from May and Baker Ltd.) is shown in Fig. 3, as it differs in some ways from that reported by Levi and his co-workers.[5,6] The spectrum of the treated disc was the same, but for an additional weak, yet sharp and distinctive, peak at 2155 cm^{-1}. A slight decrease in the sharpness of the spectrum was restored when the disc had been kept for 1 month.

SANDOPTAL—

The spectra of duplicate discs containing Sandoptal (5-allyl-5-*iso*butylbarbituric acid, melting-point 140° C) showed different degrees of sharpness after the discs had been heated, but were otherwise the same as that shown in Fig. 3.

SECOBARBITONE—

The spectrum of discs containing secobarbitone (5-allyl-5-(α-methyl)butylbarbituric acid, melting-point 88° C) after treatment is shown in Fig. 3; after 1 month the spectrum was unchanged.

THIAMYLAL—

The spectrum of discs containing Thiamylal (5-allyl-5-(α-methyl)butylthiobarbituric acid, melting-point 138° C) after treatment and the regular spectrum to which it changes after the discs have been kept for some time are shown in Fig. 3.

THIOPENTAL—

The spectrum of the treated discs containing Thiopental (5-ethyl-5-(α-methyl)butylthiobarbituric acid, melting-point 161° C) was the same as that of the crystalline compound.[7] There was slight sharpening of the spectrum after the discs had been kept for a time.

DISCUSSION OF THE METHOD

Experiments with molten films of barbiturate on heated plates of sodium chloride showed that, with some compounds, clear glasses were obtained on cooling. The spectra of these glasses were the same smooth, comparatively featureless curves as those obtained when discs of potassium bromide containing the same compounds were heated. Some did

crystallise in time, but other glasses were more stable. The spectra of barbiturate glasses, although having fewer sharp peaks than those of the crystalline forms, can still be used for identifying barbiturates. The spectra of Butethal and amobarbitone glasses are almost identical, but the melting-points of these compounds are separated by 30° C.

When purifying polymeric barbiturates, the rate of sublimation or the solvent used for recrystallisation and the conditions under which the crystals form from solution were found to influence the crystalline modification obtained and hence the spectrum; mixtures of crystalline forms were often obtained. Glasses, which are produced when fused samples do not readily crystallise, have different spectra. The grinding of solid barbiturates during preparation of the sample can change unstable forms. However, by the proposed method of heating a sample prepared in the form of a potassium bromide disc to a temperature higher than the melting-point of the sample and recording the spectrum immediately afterwards, the initial state of the sample and its subsequent treatment do not influence the spectrum obtained.

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Correction of Fluorescence Spectra and Measurement of Fluorescence Quantum Efficiency

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Methods are described for calculating true fluorescence emission and excitation spectra, and correction curves for the authors' equipment are presented. The advantages of using "true" spectra are discussed, and the use of true emission spectra to determine fluorescence quantum efficiencies is described. The "fluorescence sensitivity" of most substances can be defined completely from the fluorescence efficiency, the true fluorescence emission spectrum and the absorption spectrum.

It is proposed that published data obtained by spectrofluorimetry should whenever possible be presented in a standard form, so as to be of maximum value to other workers. Results are provided for six substances that fluoresce in the visible region.

Now that commercial spectrofluorimeters are coming into common use, the number of fluorescence spectra appearing in the literature is likely to increase rapidly and it is most desirable to get some general agreement on the method of reporting results, so that they will be of maximum value to other workers. All commercial spectrofluorimeters at present available record "apparent fluorescence emission spectra" and "apparent fluorescence excitation spectra," both of which, in some regions of the spectrum, are grossly distorted versions of the true spectra. Although for one instrumental set-up the results will be reproducible and can therefore be used directly for analytical work without correction, they are likely to differ considerably from the results obtained in another laboratory with an instrument

of a different type. It is clearly desirable that published spectra should either be corrected or reference made to a correction curve for the particular instrument and the experimental conditions employed.

The methods of determining true spectra are referred to in earlier papers.[1,2] These methods are well known to physical chemists dealing with fundamental studies of fluorescence, but they are not so familiar to analytical chemists generally. A more detailed description of the methods of correction is therefore presented in this paper; correction curves for some of the authors' equipment are recorded and the relationship between apparent and true spectra is discussed.

Once the true emission spectrum has been determined, it can be used directly to obtain the fluorescence quantum efficiency of a compound by comparison with a standard substance of known quantum efficiency. The subject of standard substances and the "fluorescence sensitivity" of any particular substance is an important one, since all quantitative spectrofluorimetric measurements rely ultimately on the comparison of the intensity of the fluorescence of the test solution with that of a solution of a standard substance. As will be seen, the "fluorescence sensitivity" can be defined by the fluorescence emission spectrum, the fluorescence quantum efficiency and the absorption spectrum of the compound. Defined in this way the "fluorescence sensitivity" of a compound is independent of the instrument used to measure it and does not rely on comparison with a standard substance. The sensitivity obtainable with a particular instrument will naturally depend on various instrumental factors, and it can be calculated from the "absolute fluorescence sensitivity" of the substance being dealt with once the correction curves for the instrument have been determined.

Origin of fluorescence spectra

The mechanism of the light absorption and emission by solutions is described in textbooks.[3,4] A brief outline is given here to show how the important rules of spectrofluorimetry can be derived and to indicate the significance of the fluorescence excitation spectrum. The arrangement of energy levels in a typical molecule is shown schematically in Fig. 1.

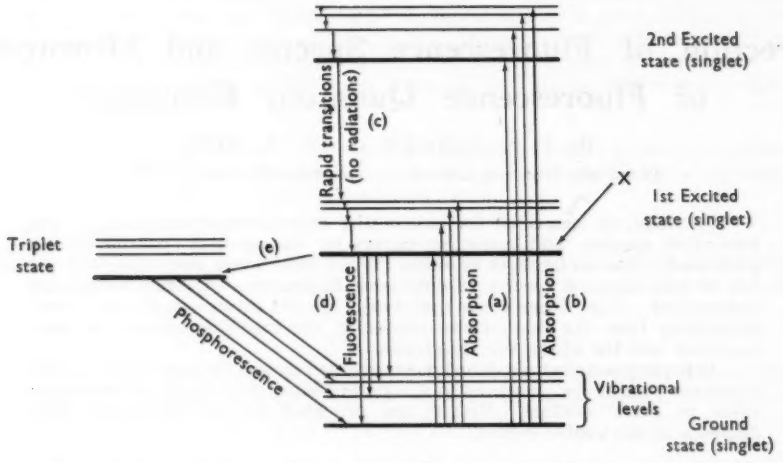

Fig. 1. Transitions giving rise to absorption and fluorescence emission spectra

Absorption of light raises the molecule from the ground state to one of the upper electronically excited states. At room temperature most molecules are in the lowest vibrational level of the ground state and it is from here that transitions upwards by absorption of light take place (transitions (a) and (b) in Fig. 1). For some molecules, such as anthracene, the pattern of vibrational levels is comparatively simple, and they appear in the absorption spectrum as well separated bands (see Fig. 2). For many other organic compounds with which one has to deal in practice the pattern of vibrational levels is more complex and all the transitions (a) to the various levels of the first excited state appear as one broad absorption band, another

broad absorption band appearing at shorter wavelengths corresponding to the transitions to the various vibrational levels of the second excited state. It so happens that for almost all complex molecules, after excitation to any condition higher than the lowest vibrational level of the first excited state, the molecule rapidly drops back to this lowest level (level X in Fig. 1)

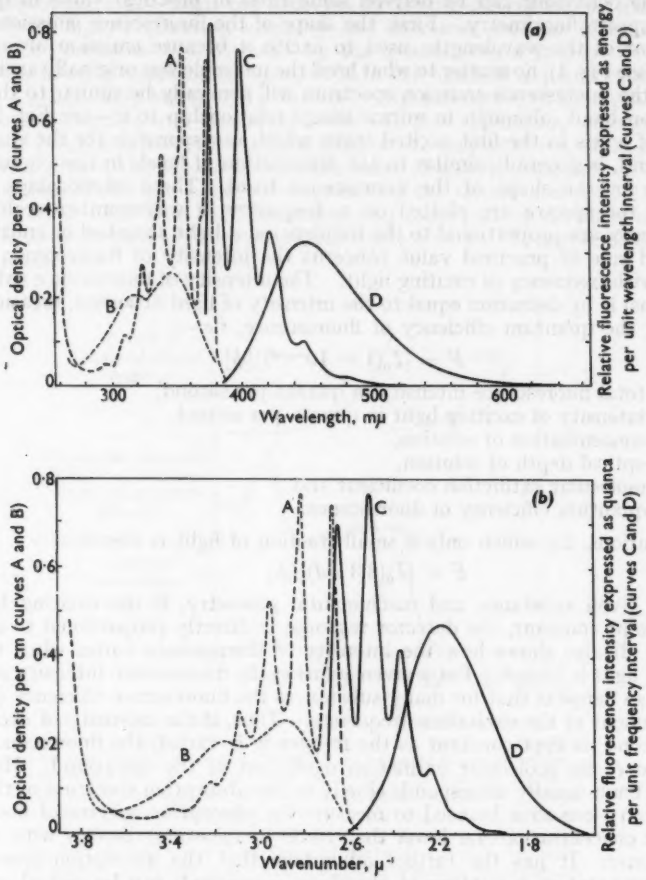

Fig. 2. Absorption and fluorescence emission spectra of anthracene and quinine bisulphate illustrating the difference between plotting in units of (*a*) wavelength and energy and (*b*) wavenumber and quanta: curve A, absorption spectrum of anthracene in ethanol (17·2 μg per ml); curve B, absorption spectrum of quinine bisulphate in 0·1 *N* sulphuric acid (25 μg per ml); curve C, fluorescence emission spectrum of anthracene in ethanol (1·0 μg per ml; optical density per 10 cm was 0·102 at 366 mμ); curve D, fluorescence emission spectrum of quinine bisulphate in 0·1 *N* sulphuric acid (1·0 μg per ml; optical density per 10 cm was 0·062 at 366 mμ)

Quartz-prism spectrometers were used for recording all spectra. For fluorescence the half-band width was 0·028 μ^{-1} (4·5 mμ) at 2·5 μ^{-1} (400 mμ); for absorption spectra the band width varied from 0·0004 μ^{-1} at 3·2 μ^{-1} to 0·0006 μ^{-1} at 1·6 μ^{-1}

by processes that do not involve the emission of light (transitions (c) in Fig. 1). (This assumes that the molecule is not excited to a level in which it undergoes photochemical decomposition.) From the level X the molecule can then return to any one of the vibrational levels of the ground state with the emission of fluorescence (transitions (d) in Fig. 1). If all the molecules originally excited return to the ground state by transition (d), the solution fluoresces with

a quantum efficiency of unity. A proportion of the excited molecules may return to the ground state by other mechanisms, for example, by conversion to the triplet state(transitions (e) in Fig. 1) or by various other de-activation processes, or some may undergo photochemical change. The fluorescence quantum efficiency is then less than unity and may be zero.

From this behaviour can be derived some rules of practical value in qualitative and quantitative spectrofluorimetry. First, the shape of the fluorescence emission spectrum will be independent of the wavelengths used to excite it because emission always takes place from level X (see Fig. 1), no matter to what level the molecule was originally excited. Secondly, the shape of the fluorescence emission spectrum will generally be similar to the shape of the first absorption band (although in mirror image relationship to it—see Fig. 2) because the distribution of levels in the first excited state, which is responsible for the shape of the first absorption band, is generally similar to the distribution of levels in the ground state, which is responsible for the shape of the fluorescence band. These relationships will naturally apply only if the spectra are plotted on a frequency or wavenumber scale, because the energy differences are proportional to the frequencies of light absorbed or emitted.

The third rule of practical value concerns the intensity of fluorescence obtained and its variation with frequency of exciting light. The intensity of fluorescence (which is emitted in all directions) is by definition equal to the intensity of light absorbed, measured in quanta, multiplied by the quantum efficiency of fluorescence, *i.e.*—

$$F = [I_0(1 - 10^{-\epsilon cd})][\phi] \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where F = total fluorescence intensity in quanta per second,
I_0 = intensity of exciting light in quanta per second,
c = concentration of solution,
d = optical depth of solution,
ϵ = molecular extinction coefficient and
ϕ = quantum efficiency of fluorescence.

For dilute solutions, for which only a small fraction of light is absorbed—

$$F = [I_0(2{\cdot}3\,\epsilon cd)][\phi] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Thus for any given substance and instrumental geometry, if the exciting frequency and intensity are kept constant, the detector response is directly proportional to concentration.

Equation (2) also shows how the intensity of fluorescence varies when the frequency of the exciting light is varied. For a given solution the fluorescence intensity is proportional to $I_0\epsilon\phi$ and it so happens that for many substances the fluorescence efficiency (ϕ) is approximately independent of the excitation frequency. Thus, if the intensity of exciting light, in quanta per second, is kept constant as the frequency is varied, the fluorescence intensity is proportional to ϵ, the molecular extinction coefficient of the compound. Hence the true excitation spectrum usually corresponds closely to the absorption spectrum of the compound. Spectrofluorimetry can thus be used to measure the absorption spectra of fluorescent compounds, but at concentrations far lower than could be measured directly with an absorption spectrophotometer. It has the further advantage that the absorption spectrum of one fluorescent component of a mixture of absorbing compounds can be picked out simply by tuning to the appropriate fluorescence band. Examples of these applications have been given in earlier papers.[1,5]

All of these rules apply only to solutions of a single substance. Thus if the fluorescence emission spectrum of a solution varies with excitation frequency or if the excitation spectrum differs markedly from the absorption spectrum, the presence of more than one component should be suspected, although there are some exceptional substances to which the above rules do not apply.

Measurement of fluorescence excitation spectrum

To measure the excitation spectrum the intensity of the fluorescence band (or a part of it isolated by a monochromator or filters) is recorded as a function of the wavelengths or frequency setting of the monochromator used to provide the monochromatic exciting light. The apparent excitation spectrum so obtained is a plot of the product $I_0\phi\epsilon$ against frequency or wavelength. To obtain the true excitation spectrum the intensity of the exciting light, I_0, must be determined as a function of frequency. This may be done by means of a sensitive thermopile, a calibrated phototube, the ferrioxalate actinometer or a fluorescent screen of

constant quantum efficiency.[5] The thermopile reading gives the relative intensities in energy units (E), and each reading must be divided by the frequency (or multiplied by the wavelength) to give relative intensities in terms of quanta. Similarly, phototube calibration curves provided by the manufacturers are frequently given in microwatts and must similarly be converted to units of quanta. With the actinometer or the fluorescent screen the results obtained are proportional to quantum intensity and therefore need no further correction.

The method of correction is illustrated by the results shown in Fig. 3. The upper curve, A, shows the relative quantum intensity of light delivered by a quartz monochromator (with wide slits), a xenon lamp being used as source. The full curve, B, shows the absorption spectrum of quinine sulphate and the lower curve, C, the apparent excitation spectrum. Owing to the rapidly falling intensity of exciting light as the frequency increases, the apparent excitation spectrum shows the second absorption band of quinine sulphate at an intensity much lower than that of the first band, whereas in fact the second absorption band is considerably more intense. The true excitation spectrum (obtained by dividing the ordinates of curve C by those of curve A) is shown as circles on curve B. It agrees closely with the absorption spectrum.

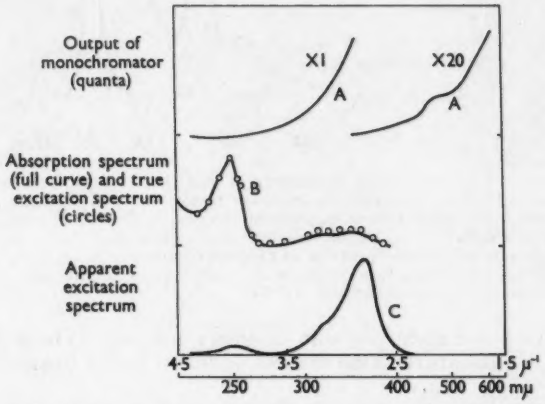

Fig. 3. Comparison of absorption and excitation spectra of quinine bisulphate in 0·1 *N* sulphuric acid: curve A, relative quantum intensity of light delivered by a quartz monochromator, with wide slits, a xenon lamp being used as source; curve B (full line), absorption spectrum of quinine bisulphate solution (relative optical density); curve B (circles), true excitation spectrum of quinine bisulphate solution obtained by dividing the ordinates of curve C by those of curve A; curve C, apparent excitation spectrum of quinine bisulphate solution (fluorescence intensity as a function of frequency setting of excitation monochromator at constant slit width)

The unfavourable distribution of ultra-violet sources can sometimes obscure important features of the excitation spectrum unless corrections are applied. For example, the absorption spectrum of alkaline fluorescein solution shows an intense band in the visible region and three quite prominent bands in the ultra-violet region (see full curve A, Fig. 4). The apparent excitation spectrum shows only the band in the visible region, and it is necessary to increase the sensitivity by a factor of 100 to show the ultra-violet part of the spectrum, and even then it is greatly distorted (see curve B, Fig. 4). After correction to constant exciting intensity to give the true excitation spectrum (circles on curve A in Fig. 4) the results agree well with the absorption curve.

The correction of excitation spectra can be tedious, particularly with complicated spectra, and it is a great advantage to have a spectrofluorimeter that records directly the true excitation spectrum. This can be done by using a split-beam arrangement and ratio recorder, in which the exciting light is monitored by means of a fluorescent screen of constant quantum efficiency.[5]

Measurement of fluorescence emission spectrum

With a constant intensity of exciting light of the chosen frequency, the photomultiplier response is recorded at constant slit width as a function of the frequency setting of the monochromator used to analyse the fluorescence. This gives the apparent fluorescence emission spectrum. The true fluorescence emission spectrum is a plot of intensity of

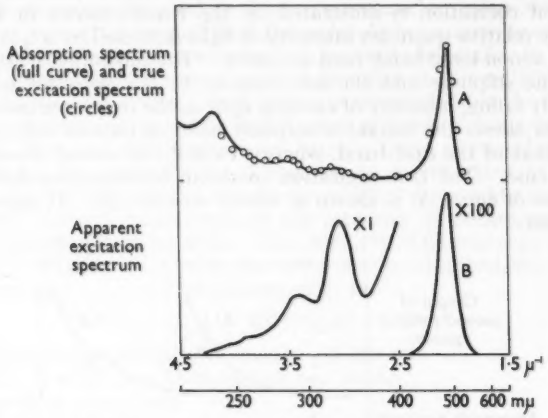

Fig. 4. Comparison of absorption and excitation spectra of fluorescein in carbonate - bicarbonate buffer: curve A (full line), absorption spectrum of fluorescein solution; curve A (circles), true excitation spectrum of fluorescein solution; curve B, apparent excitation spectrum of fluorescein solution (fluorescence intensity as a function of frequency setting of excitation monochromator at constant slit width)

fluorescence, expressed as quanta per unit frequency interval, against frequency or wavenumber. Thus if Q represents the total number of quanta (of all frequencies) of fluorescence emitted per unit time, then $\frac{dQ}{d\nu}$ represents the intensity at any frequency, ν, and the plot of $\frac{dQ}{d\nu}$ against ν is the true fluorescence emission spectrum. Integration of this curve will give the total quanta, Q, emitted, which is required for calculating the absolute quantum efficiency of fluorescence (see below). In practice, the spectrum is plotted in relative rather than absolute units, and we require to know the relationship between this true relative emission spectrum and the apparent spectrum recorded by the instrument. The difference between the two is accounted for by three factors, namely, the variation in quantum sensitivity of the photomultiplier with frequency, the varying band width of the spectrometer and the light losses within the spectrometer. Thus the observed photomultiplier output at any frequency, A_ν, which corresponds to the apparent emission spectrum, is given by—

$$A_\nu = \left(\frac{dQ}{d\nu}\right)(B_\nu L_\nu P_\nu) = \left(\frac{dQ}{d\nu}\right)(S_\nu)$$

where P_ν = output per quantum of photomultiplier at frequency ν,
B_ν = band width in frequency units at frequency ν and
L_ν = fraction of light transmitted by the spectrometer at frequency ν.

The quantity S_ν, equal to $B_\nu L_\nu P_\nu$, is the sensitivity factor of the monochromator - photomultiplier combination, and the true emission spectrum is derived from the apparent emission spectrum by dividing by S_ν. S_ν is clearly proportional to the photomultiplier output that would be observed when a source of constant spectral distribution $\left(\frac{dQ}{d\nu}\text{ constant}\right)$ is

used. If, therefore, a lamp of known spectral distribution is placed in line with the entrance slit of the spectrometer and the photomultiplier response (R_{SL}) recorded as a function of frequency, then S_ν can simply be determined from—

$$S_\nu = \frac{R_{SL}}{(dQ/d\nu)_{SL}}$$

This is the method generally used for calibrating in the visible region. Tungsten lamps can be calibrated, *e.g.*, by the National Physical Laboratory, to run at a known colour temperature, from which can be calculated the spectral distribution of the light emitted. The data are normally provided in the form of energy units (microwatts) per unit wavelength interval, *i.e.*, $\frac{dE}{d\lambda}$. They must first be multiplied by the corresponding wavelengths to give quanta per unit wavelength interval, *i.e.* $\left(\frac{dE}{d\nu}\right) . \lambda$. They must then be converted to quanta per unit frequency interval by multiplying again by λ^2. Thus the equation for conversion is—

$$\left(\frac{dQ}{d\nu}\right)_{SL} = \left(\frac{dE}{d\lambda}\right)_{SL} . \lambda^3$$

The λ^2 factor arises from the relationship—

$$\nu = \frac{c}{\lambda}$$

and hence—

$$\left(\frac{dQ}{d\nu}\right) = \left(\frac{dQ}{d\lambda}\right)\left(\frac{d\lambda}{d\nu}\right) = -\left(\frac{dQ}{d\lambda}\right) . \frac{\lambda^2}{c}$$

Hence the sensitivity factor, S_ν, is given by—

$$S_\nu = \frac{R_{SL}}{\left(\frac{dE}{d\lambda}\right)_{SL} . \lambda^3} .$$

The various stages in the calculation are shown in Fig. 5 for a glass-prism monochromator and red-sensitive photomultiplier. Since the figures at the various stages of the calculation are in relative units only, all curves have been normalised to give the same reading at 560 mμ.

Unfortunately, lamps with accurately known spectral distribution in the ultra-violet region cannot be purchased at present, and the correction curve must be calculated directly from the relationship—

$$S_\nu = B_\nu L_\nu P_\nu$$

The photomultiplier sensitivity curve is usually supplied by the manufacturer. It can be checked by comparision with a thermopile or chemical actinometer. The band width, B_ν, can be simply calculated from the dispersion data of the prism material and is usually given by the manufacturer in the form of a curve showing band width in mμ per unit slit width, *i.e.*, $B\lambda$, which for grating instruments is constant. The light loss within the monochromator, L_ν, is rather more difficult to measure accurately. Although it may vary considerably between the visible region and the far end of the quartz ultra-violet region, it will generally be approximately constant over the restricted spectral region covered by one fluorescence band. On this assumption, the sensitivity curve can be calculated from—

$$S_\nu = \left(\frac{B\lambda}{\lambda^2}\right)\left(\frac{P_E}{\lambda}\right)$$

where the band width is given in wavelengths ($B\lambda$) and the photomultiplier sensitivity is expressed in energy units (P_E). The sensitivity curve for a combination of quartz monochromator and E.M.I. 6256B ultra-violet-sensitive photomultiplier is shown in Fig. 6, curves A and B. The results in the visible region obtained with the standard lamp agree reasonably well with those calculated by the above method over the region where both sets of results are available. The sensitivity of the ultra-violet-sensitive photomultiplier falls rapidly below

about 1·9 μ^{-1} and for light of wavenumber $<1{\cdot}8\ \mu^{-1}$ the alternative photomultiplier-monochromator combination (see curve C) has to be used. With this, reasonable results can be obtained down to about 1·5 μ^{-1}.

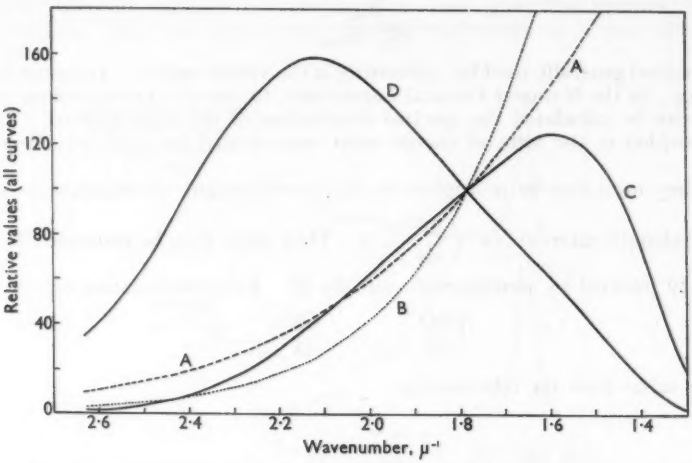

Fig. 5. Calculation of sensitivity curve for the visible region (combination of glass-prism spectrometer and E.M.I. 9558 red-sensitive photomultiplier): curve A, relative spectral distribution of light from standard lamp run at a colour temperature of 2856° K (energy per unit wavelength interval—dE/dλ); curve B, relative spectral distribution of light from standard lamp run at a colour temperature of 2856° K (quanta per unit frequency interval—dQ/dν); curve C, photomultiplier output at constant slit width; curve D, sensitivity curve, Sν (ordinates of curve C divided by ordinates of curve B)

All curves normalised to 0·56 μ

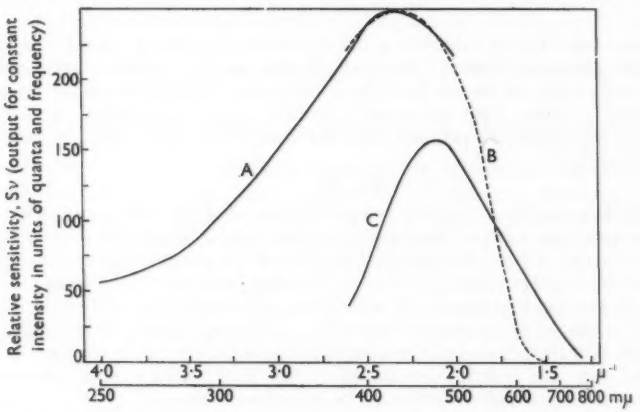

Fig. 6. Sensitivity curves in the ultra-violet and visible regions: curve A, combination of quartz-prism spectrometer and E.M.I. 6256B photomultiplier —results calculated from the relationship $S\nu = B\nu P\nu$; curve B, as for curve A, but determined by comparison with standard lamp; curve C, combination of glass-prism monochromator and E.M.I. 9558 photomultiplier

Measurement of fluorescence quantum efficiency

The integrated area under the fluorescence emission spectrum is proportional to the total intensity of fluorescent light emitted by the solution, and this in turn is proportional to the product $I_0\phi\epsilon cd$. Thus, if the fluorescence emission spectra of two solutions are measured

with the same apparatus and at the same exciting intensity, I_0, the ratio of the two fluorescence intensities is given by—

$$\frac{F_2}{F_1} = \frac{\text{area } 2}{\text{area } 1} = \frac{\phi_2 E_2 c_2 d}{\phi_1 E_1 c_1 d} = \left(\frac{\phi_2}{\phi_1}\right)\left(\frac{\text{optical density of } 2}{\text{optical density of } 1}\right)$$

If the optical densities of the two solutions are measured, the ratio of the fluorescence quantum efficiencies can be derived. If one of the substances chosen has a known fluorescence quantum efficiency, the value of the other is then simply obtained. In practice, it is most convenient to record separately the two apparent emission spectra. The true spectra are then calculated, the areas under the curves measured, and the ratio "area to peak height" is calculated. The two solutions are then accurately compared by measuring the fluorescence intensities at their respective maxima.

Some relative fluorescence efficiencies measured at various exciting frequencies are shown in Table I. With the exception of quinine sulphate (B.D.H. laboratory-reagent grade) and anthracene (B.D.H. "blue fluorescent") the compounds had been recrystallised several times

TABLE I

RELATIVE FLUORESCENCE QUANTUM EFFICIENCIES

Substances compared	Relative fluorescence efficiency with exciting light of—				
	2·73 μ^{-1} (366 mμ)	2·29 μ^{-1} (436 mμ)	2·14 μ^{-1} (467 mμ)	2·03 μ^{-1} (492 mμ)	1·83 μ^{-1} (546 mμ)
F/R	1·38*	1·32*	1·23	1·23	—
E/R	0·34	0·35*	0·33	0·31	—
Q/R	0·80	—	—	—	—
T/R	—	—	—	—	0·035
A/Q	0·516	—	—	—	—

F = Fluorescein in 0·1 *N* sodium hydroxide; approximate concentrations, 1·5 μg per ml (366 and 436 mμ) and 0·15 μg per ml (467 and 492 mμ).

E = Eosin in 0·1 *N* sodium hydroxide; approximate concentrations, 2·5 μg per ml (366, 436 and 467 mμ) and 1·25 μg per ml (492 mμ).

R = Rhodamine B in ethanol; approximate concentrations, 2·3 μg per ml (366, 436 and 467 mμ), 1·2 μg per ml (492 mμ) and 0·012 μg per ml (546 mμ).

Q = Quinine bisulphate in 0·1 *N* sulphuric acid; approximate concentration, 1·0 μg per ml (366 mμ).

A = Anthracene in ethanol; approximate concentration, 1·0 μg per ml (366 mμ).

T = Thionine hydrochloride in 0·1 *N* sulphuric acid; approximate concentration, 0·6 μg per ml (546 mμ).

* These results were less accurate owing to low absorption of fluorescein in 0·1 *N* sodium hydroxide at 366 mμ and of rhodamine B in ethanol at 436 mμ.

and were considered to be free from fluorescing or absorbing impurities. By using Melhuish's value,[6] 0·55, for the absolute fluorescence efficiency of quinine bisulphate, the results in Table I were used to derive absolute fluorescence efficiencies for the other five compounds (see Table II). With the exception of that for rhodamine B, the results agree reasonably well with the literature values.[7,8,9,10,11] The determination of absolute quantum efficiency directly, without reference to a standard substance of known quantum efficiency, is a difficult matter and is subject to many sources of error, as can be judged from the different values quoted in the past for the same substance. The relative values obtained by the method described above are probably more accurate than any single absolute determination.

Some of the substances quoted in Tables I and II have been investigated as possible standards for fluorimetric measurement. It was found that alkaline solutions of eosin were unsuitable for this purpose owing to rapid decomposition with the formation of products absorbing more strongly at 2·73 μ^{-1} (366 mμ). Fluorescein was found to be more stable, particularly in carbonate - bicarbonate buffer at a pH of about 9·6, for which the absorption and fluorescence spectra and the fluorescence efficiency were identical with those observed in 0·1 *N* sodium hydroxide. However, its absorption at 2·73 μ^{-1} (366 mμ) is low, so that fairly large errors can be introduced by the presence of traces of impurity absorbing at this wavelength. It is more useful for the lower frequencies, *e.g.*, 2·29 μ^{-1} (436 mμ). Rhodamine B in ethanol is also stable and shows higher absorption at 2·73 μ^{-1} (366 mμ), although a low

absorption at 2·29 μ^{-1} (436 mμ). Quinine bisulphate also appears to be a suitable standard substance, although for comparison between different laboratories it is desirable to quote the quinine content of the sample used or alternatively to express concentrations in terms of optical density at 2·73 μ^{-1} (366 mμ). (The sample used in the work described gave an optical density at 2·73 μ^{-1} of 0·0062 per cm for a concentration of 1 μg per ml.) The choice of standard substances for spectrofluorimetry will undoubtedly widen rapidly as experience with the technique in different laboratories accumulates, and it would be desirable to have a choice of standard substances and a method of expressing sensitivity that was generally accepted.

TABLE II

FLUORESCENCE QUANTUM EFFICIENCIES BY REFERENCE TO QUININE BISULPHATE*

Solution	Fluorescence efficiency (ϕ)	Literature values (direct determinations)	Reference No.†
Quinine bisulphate in 0·1 *N* sulphuric acid	[0·55]	0·55	6
Rhodamine B in ethanol	0·69	0·97	7
Fluorescein in 0·1 *N* sodium hydroxide ..	0·85	0·92	7
		0·78	8
		0·84	9
		0·85	10
		0·85	11
Eosin in 0·1 *N* sodium hydroxide	0·23	0·19	7
Anthracene in ethanol	0·28	0·26‡	6
Thionine in 0·1 *N* sulphuric acid	0·024	—	—

* See Table I.
† See reference list, p. 600.
‡ Estimated from the recorded value of 0·22, which was uncorrected for self-absorption.

CHOICE OF UNITS

It may be argued that for practical analytical work the somewhat laborious correction of fluorescence spectra point by point is not worthwhile, since apparent spectra obtained on one particular instrument will be reproducible. This is certainly a legitimate argument for routine work, and uncorrected spectra are even acceptable for publication, provided that the appropriate correction curve is also available in the literature. On the assumption that a correction curve is to be provided, the choice of units must be decided. The correction curve for excitation spectra is simply the relative intensity distribution of the exciting light reaching the fluorimeter cell as a function of the frequency setting of the excitation monochromator. The relative intensity must of course be expressed in units of quanta, and not energy, for the reasons given earlier. For the fluorescence emission correction curve there are at least three good reasons for using units of quanta per unit frequency interval rather than energy per unit wavelength interval (a system some workers seem to have adopted[2]). First, with the latter system the fluorescence spectrum obtained is a distorted version not bearing the correct relationship to the corresponding absorption band. Secondly, if wavelength rather than frequency is used for the horizontal scale, the bands are unnaturally bunched together in the short-wavelength region, an undue amount of space being given to the long-wavelength region where there is less information available. Both these effects can be seen by comparing the two halves of Fig. 2, where, on the energy - wavelength system, the fluorescence bands at longer wavelengths are reduced in intensity and extended in width compared with those at the shorter wavelengths. Thirdly, if energy units rather than quanta are used, the integrated area under the fluorescence spectrum is not proportional to the fluorescence efficiency, since the quanta of lower frequency carry proportionately less energy. Thus, for two solutions having the same fluorescence efficiency, that having its fluorescence band at the longer wavelengths will give the lower energy yield.

There are practical considerations to be taken into account. For example, grating instruments produce directly a record that is linear in wavelength, and with these there is some justification for plotting spectra in terms of quanta per unit wavelength interval (the integrated area again being proportional to quantum efficiency). On the other hand, with prism instruments there is no justification for recording in terms of wavelength (other than that the manufacturers normally provide calibration in wavelength and most users are more accustomed to it) because the scale is generally more nearly linear in frequency. With regard

to the frequency unit to be employed, the authors favour the reciprocal micron (μ^{-1}), a convenient unit for the visible and ultra-violet regions (1·0 to 5·0 μ^{-1} corresponds to 1000 to 200 mμ).

Methods of expressing sensitivity

The intensity of fluorescence, of all frequencies, emitted by a dilute solution of one substance irradiated by light of a particular frequency is proportional to $I_0\phi\epsilon cd$—see equation (2). The product $\phi\epsilon$ is characteristic of the substance at the chosen frequency for excitation and is a measure of its fluorescence sensitivity to this frequency. For most substances (for which ϕ is substantially independent of frequency of excitation), the maximum sensitivity is obtained at the peak of the most intense absorption band. (The observed sensitivity with a particular instrument will not necessarily be maximum at this point because the intensity of exciting light available may be much greater at some other frequency—see, for example, Fig. 3). A convenient practical unit for ϵ is optical density per cm for a concentration of 1 μg per ml and on this basis the maximum sensitivity values of the substances in Tables I and II have been calculated (see column 5 of Table III). The sensitivity for any other excitation frequency can of course be derived directly from the maximum values by reference to the absorption spectrum (or, more strictly, the excitation spectrum).

Table III

Absolute fluorescence sensitivities

Fluorescence efficiencies are taken from Table II
$D_{max.}$ = optical density per cm for a concentration of 1 μg per ml at the main absorption peak
D_{366} = corresponding figure at 366 mμ (2·73 μ^{-1})

Substance	Absorption maximum, μ^{-1} (mμ)	Fluorescence maximum, μ^{-1} (mμ)	Half-band width of fluorescence spectrum (H), μ^{-1}	Fluorescence sensitivity at absorption maximum— for whole fluorescence band ($\phi D_{max.}$)	Fluorescence sensitivity at absorption maximum— for peak fluorescence $\left(\frac{\phi D_{max.}}{H}\right)$	Fluorescence sensitivity at 2·73 μ^{-1} (366 mμ) $\left(\frac{\phi D_{366}}{H}\right)$
Quinine bisulphate in 0·1 *N* sulphuric acid	4·00(250)	2·17(461)	0·47	0·031	0·066	0·0073
Rhodamine B in ethanol	1·84(544)	1·75(571)	0·17	0·15	0·88	0·049
Fluorescein in 0·1 *N* sodium hydroxide	2·04(490)	1·94(515)	0·20	0·20	1·0	0·015
Eosin in 0·1 *N* sodium hydroxide	1·93(518)	1·85(540)	0·18	0·035	0·19	0·0031
Anthracene in ethanol	3·97(252)	2·505(399)	0·25	0·32	1·3	0·011
Thionine hydrochloride in 0·1 *N* sulphuric acid	1·67(598)	1·61(621)	0·18	0·0056	0·031	0·00028

When a monochromator is used rather than filters for isolating the fluorescence, it is usual to select not the complete fluorescence emission spectrum, but a narrow band of frequencies at the peak. Under these conditions the sensitivity will depend not only on the product $\phi\epsilon$ but also on the effective half-band width (H) of the fluorescence spectrum. When comparing one substance with another the factor $\frac{\phi\epsilon}{H}$ is therefore also of interest, and this has been calculated in Table III both for the absorption maximum (column 6) and for 2·73 μ^{-1} (366 mμ) (column 7), which is frequently used in fluorimetry. Comparison between columns 6 and 7 of Table III shows that 2·73 μ^{-1} is far from being the best excitation frequency for the substances concerned, the sensitivity being some 10 to 100 times less than at the absorption maximum. The popularity of 2·73 μ^{-1} is due to the fact that it can be readily isolated at high intensity from a mercury lamp and the intensity factor may outweigh the sensitivity factor as between 2·73 μ^{-1} (366 mμ) and, for example, 3·94 μ^{-1} (254 mμ).

If all fluorescence sensitivity results were tabulated in a form similar to Table III (preferably giving all absorption and fluorescence maxima and noting any major discrepancy between the absorption and excitation spectrum) they would be of considerable value to

all users. For example, suppose the sensitivity of a particular spectrofluorimeter to quinine bisulphate with excitation at 2·73 μ^{-1} is known and the sensitivity to thionine is required; reference to Table III (column 7) shows that the sensitivity of thionine is $\frac{0{\cdot}00028}{0{\cdot}0073}$, *i.e.*, 0·038 of that of quinine bisulphate. Reference to the sensitivity curve for the instrument (*e.g.*, Fig. 6, curve C) shows that the sensitivity at the thionine fluorescence maximum, 1·61 μ^{-1}, is only 0·41 of that at the quinine maximum, 2·185 μ^{-1}. Hence the instrumental sensitivity to thionine fluorescence is 0·038 × 0·41 or 0·016 of its sensitivity to quinine bisulphate. Reference to the absorption spectrum of thionine shows that this sensitivity could be increased more than 100 times by using an excitation frequency corresponding to peak absorption (1·67 μ^{-1}). The final choice of excitation frequency would naturally depend on other factors, such as the intensitities available for excitation and the characteristics of any other components of the solution.

The sensitivity results (see Table III) discussed above are independent of the instrument used to measure them. The analytical chemist using spectrofluorimetry is also interested in another kind of sensitivity, that is, the sensitivity of his own instrument compared with those of other workers. This may be defined as the minimum detectable signal-to-noise ratio, and can be expressed in terms of a minimum detectable concentration of some standard substance with a chosen frequency for excitation.[1] It depends on the characteristics of the photomultiplier - amplifier combination, the light-gathering power of the monochromators and the intensity of the source. However, the factor limiting sensitivity is frequently not the signal-to-noise ratio but the over-all "fluorescence blank," which may include contributions from fluorescence of the cuvette or impurities in the reagents, stray light or even the Raman spectrum of the solvent. In quoting an instrumental sensitivity it should be made clear which of the two factors is limiting, and as many as possible of the variables should be quoted (*e.g.*, intensity and frequency of the exciting light, band widths of the excitation and fluorescence monochromators and, for filter fluorimetry, the purity of the exciting light and the band pass of the secondary filters). A useful discussion of the evaluation of filter fluorimeter performance has been given by Greengard.[12] Measurement of the Raman spectrum of a solvent is a useful test of the performance of a spectrofluorimeter.[13]

Precautions

In measuring relative fluorescence quantum efficiencies, as in all spectrofluorimetry, precautions must be taken to avoid errors due to inner filter effects, oxygen quenching, non-monochromatic exciting light and photo-decomposition.[1] Errors due to the first two factors are in principle simple to avoid, but in practice they are easily overlooked. Equation (2) applies only if the percentage of exciting light absorbed by the two solutions is negligible at the depth from which the fluorescence is viewed. An optical density of 0·02 at this point (approximately 4 per cent. of light absorbed) will introduce an error of about 4 per cent., and the optical density should not be allowed to exceed this value. Alternatively, if the fluorescence efficiency is so low or the instrumental sensitivity so small that high optical densities have to be employed, then to obtain the correct results the optical densities of the two solutions should be adjusted to the same value.

There is a second kind of inner filter error that can easily occur with substances such as anthracene, for which the absorption and fluorescence spectra overlap and the exciting light used (*e.g.*, 2·73 μ^{-1}) is comparatively weakly absorbed. Under these conditions if a spectrofluorimeter of low sensitivity is used, with narrow slits to resolve the anthracene bands, a comparatively high concentration must be used to get sufficient fluorescence intensity, and the self-absorption of the high-frequency end of the fluorescence band by the low-frequency end of the absorption band becomes appreciable. Such distortion of the fluorescence spectrum is shown by the curves in Fig. 7. These relate to an arrangement in which the optical path taken by the fluorescence light in emerging from the liquid was short (5 mm). With a longer optical path the self-absorption would be correspondingly greater.

Anthracene shows appreciable oxygen quenching of its fluorescence in solution (see Table IV). Saturation with air reduces the fluorescence intensity by 14 per cent. Clearly, if anthracene is to be used as a fluorescence standard precautions must be taken either to saturate the solution with air or to de-aerate it completely before measurement. Because of the common occurrence of oxygen quenching, it is always a wise precaution during the

early stages of the investigation of the fluorescence of a new compound to test qualitatively for oxygen quenching (it is negligible for the other substances listed in Table I). In exceptional instances it can reduce the fluorescence intensity by large factors.[1]

TABLE IV

QUENCHING OF FLUORESCENCE OF ANTHRACENE IN ETHANOL BY OXYGEN

There was a slight increase in concentration during the experiment owing to evaporation

Treatment of solution	Fluorescence intensity
Original solution (air saturated)	74·2
Nitrogen passed for 8 minutes	87·2
Air passed for 8 minutes	75·3
Oxygen passed for 8 minutes	46·3
Nitrogen passed for 8 minutes	89·2

Large errors can be introduced in the determination of fluorescence efficiency if the exciting light is not pure. For example, the mercury line at 3·19 μ^{-1} (313 mμ) is weakly absorbed by anthracene, the absorption of the mercury line at 3·942 μ^{-1} (253·7 mμ) being over 100 times as great. Thus if the 3·19 μ^{-1} line, isolated by filters, is used for excitation, the presence of

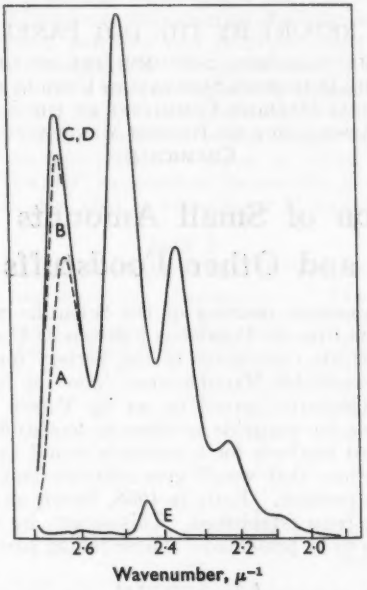

Fig. 7. Effect of self-absorption of fluorescence. Apparent emission spectra of solutions of anthracene in ethanol with excitation at 2·73 μ^{-1} (366 mμ): curve A, 17·2 μg per ml; curve B, solution diluted ×5 and measured at sensitivity ×5; curve C, solution diluted ×25 and measured at sensitivity ×25; curve D, solution diluted ×125 and measured at sensitivity ×125; curve E, ethanol only measured at same sensitivity as for curve D. (Curve D has been corrected for Raman and fluorescence emission of ethanol—curve E)

an amount of 3·942 μ^{-1} radiation in the beam amounting to only 1 per cent. of the intensity of the whole beam will be more than sufficient to double the fluorescence intensity observed. It is therefore always desirable to check the purity of the exciting light. This can easily be done by filling the cuvette with a slightly turbid solution and measuring the spectrum of scattered light with the fluorescence monochromator.

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Recommended Methods of Analysis of Pesticide Residues in Foodstuffs

REPORT BY THE DDT PANEL

SET UP JOINTLY BY THE SCIENTIFIC SUBCOMMITTEE OF THE INTERDEPARTMENTAL ADVISORY COMMITTEE ON POISONOUS SUBSTANCES USED IN AGRICULTURE AND FOOD STORAGE, THE ANALYTICAL METHODS COMMITTEE OF THE SOCIETY FOR ANALYTICAL CHEMISTRY, AND THE ASSOCIATION OF BRITISH MANUFACTURERS OF AGRICULTURAL CHEMICALS

The Determination of Small Amounts of DDT in Flour and Other Foodstuffs

IN October, 1956, a representative meeting of the Scientific Subcommittee of the Interdepartmental Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage, the Analytical Methods Committee of the Society for Analytical Chemistry and the Association of British Insecticide Manufacturers (now the Association of British Manufacturers of Agricultural Chemicals) agreed to set up Panels to undertake collaborative studies of analytical methods for pesticide residues in foodstuffs.

Published or unpublished methods for a pesticide would be examined with the aim of arriving at a detailed procedure that would give accurate and reproducible results for as wide a range of foodstuffs as possible. Early in 1958, Panels on DDT and BHC began their work, and others have since been established. This report, by the DDT Panel, is the first of these collaborative studies to be presented. Those taking part are listed in Appendix III.

Experimental

At the beginning of the investigation, analytical methods used at Long Ashton Research Station[1] and at the Laboratory of the Government Chemist[2] were considered. Both these methods were based on that originally described in 1945 by Schechter and his co-workers.[3] A composite method devised from these procedures was used in a collaborative study of a carbon tetrachloride extract of grain to which a definite amount of DDT, unknown to the individual analysts, had been added. However, owing to variations in technique, the results were not suitable for statistical analysis.

Difficulties were experienced with the use of permanganate oxidation for "cleaning-up" fatty materials, and this procedure was abandoned in favour of a column of the type described by Davidow.[4] Results were still variable, and it was shown that aromatic impurities in the light petroleum used could lead to loss of DDT; the use of *n*-hexane was proposed. Further work was then carried out, the members themselves adding DDT to wholemeal flour, with fairly good results.

At this stage, experience had shown that errors could occur—

(*i*) during permanganate oxidation,

(*ii*) owing to aromatic impurities in light petroleum or *n*-hexane and

(*iii*) owing to loss of DDT from the column of silica gel unless the gel was specially selected.

Losses during permanganate oxidation had been eliminated, as the oxidation had been replaced by use of an acid - Celite column. Methods were laid down for purifying light petroleum and *n*-hexane and are described in Appendix II. (It should be noted that the reagent described as "petroleum spirit, 'AnalaR,' free from aromatic hydrocarbons" may contain up to 0·5 per cent. of such hydrocarbons.) The *n*-hexane used was of laboratory-reagent grade, this being the purest grade available. The method of analysis was then revised to cover the above points. In principle it consisted in extracting the DDT from the foodstuff with a solvent, usually *n*-hexane or *n*-hexane and acetone (but sometimes light petroleum), cleaning-up the extract on the acid - Celite column and then on a silica-gel column, nitrating the cleaned-up extract and determining the tetranitro-DDT by measuring the blue colour developed by its benzene solution on addition of ethanolic potassium hydroxide.

Results

In Table I are shown the results of collaborative determinations by the recommended method (see Appendix I) of DDT added to 20-g samples of wholemeal flour. In experiment A, the pesticide was added by direct weighing and in experiments B and C it was added as a standard solution in carbon tetrachloride. In experiment E, to cover a wider range of residues, it was decided to circulate a sample containing at least 10 p.p.m. of added DDT. The concentration chosen was 12·5 p.p.m., and, so that analysts could check the concordance of their results on aliquots of an extract, sufficient of the sample was circulated to permit the extract to be halved. A sample containing 13·7 p.p.m. of added DDT was circulated at the same time (experiment D). The DDT contents of the samples in experiments A, B, C and E were unknown to the analysts, but that in experiment D was declared in advance.

Table I

Summary of collaborative results for DDT in wholemeal flour

Laboratory No.	Experiment A		Experiment B		Experiment C		Experiment D		Experiment E	
	DDT added, p.p.m.	DDT found, p.p.m.	DDT added, p.p.m.	DDT found, p.p.m.	DDT added, p.p.m.	DTD found, p.p.m.	DDT added, p.p.m.	DDT found, p.p.m.	DDT added, p.p.m.	DDT found, p.p.m.
1	4·6	3·4	2·5	1·5	5·0	4·0	13·7	12·7	12·5	10·6, 11·5
	3·4	1·6	—	—	—	—	—	—	—	—
2	9·0	6·3	2·5	2·5	5·0	4·8	13·7	13·5	12·5	12·2, 12·4
	4·8	6·0	—	—	—	—	—	—	—	—
3	4·5	4·4	2·5	3·9	5·0	6·5	—	—	—	—
4	5·6	5·2	—	—	—	—	—	—	12·5	11·1, 12·3
	5·0	5·0	—	—	—	—	—	—	—	—
5	10·0	10·1	2·5	3·4	5·0	6·3	13·7	13·4	12·5	13·3, 13·2
	11·0	7·9	—	—	—	—	—	—	—	—
6	5·9	8·0	—	—	—	—	13·7	12·5	12·5	9·9, 10·4
	4·5	4·4	—	—	—	—	—	—	—	—
7	4·2	3·5	—	—	—	—	—	—	—	—
	2·5	3·6	—	—	—	—	—	—	—	—
8	6·6	6·6	—	—	5·0	4·7	—	—	—	—
	5·8	5·5	—	—	—	—	—	—	—	—
9	—	—	2·5	3·4	5·0	6·2	13·7	13·4	12·5	12·6
Mean	..	.. ..	..	2·94		5·42		13·10		11·77
Standard deviation		±2·5		±0·90		±1·0		±0·46		±1·13
Coefficient of variation, %		26·7		32·4		18·5		3·5		9·7

Although the variation was greater for the samples of unknown DDT content than for the known, the Panel considered that it was not unreasonable for a method of this kind.

The work reported has been confined to wholemeal flour, but individual members of the Panel have satisfactorily used the method described in Appendix I, with the indicated modifications to the extraction procedure, for other commodities. As work with flour has shown that reasonable agreement is possible between different workers once an extract has been obtained, the Panel recommends this method for use.

Appendix I

RECOMMENDED METHOD FOR DETERMINING SMALL AMOUNTS OF DDT IN FLOUR AND OTHER FOODSTUFFS

APPARATUS—

All-glass apparatus should be used throughout.

Chromatographic tube—A filter tube, 30 mm × 200 mm, fitted with a No. 1 sintered-glass plate.

Tube for silica gel—A glass tube, 16 mm × 200 mm, tapering at the lower end to retain a cotton-wool plug.

Evaporator—This is a simplified form of the Kuderna - Danish evaporator and is shown in Fig. 1 (*b*). It consists of a 500-ml round-bottomed flask, the neck of which is fitted with a B24 socket and to the bottom of which is sealed a B14 cone. In use, a splash head leading to a condenser for solvent recovery is fitted to the socket, and a 25-ml round-bottomed flask with a B14 ground-glass socket is attached to the cone and held in place by a spring clip. The apparatus is heated by supporting the larger flask on an open-ended cylinder, *e.g.*, made from a tin can, that stands on a steam-bath in a way such that the 25-ml flask and the lower part of the larger flask are surrounded by steam. A few glass beads or a narrow strip of polytetrafluoroethylene sheeting is necessary to prevent the boiling solvent from bumping.

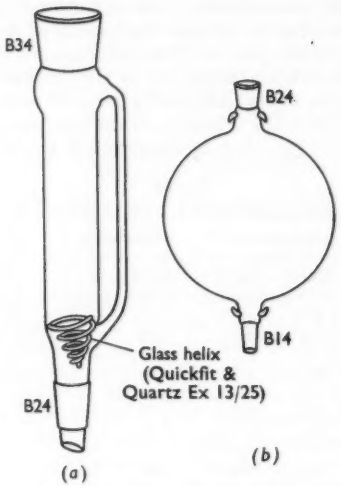

Fig. 1. (*a*) Modified Quickfit & Quartz FC8/23 extraction apparatus; (*b*) Kuderna - Danish evaporator

Extraction apparatus—A large Soxhlet extractor or a percolator consisting of a fractionating column (Quickfit & Quartz FC8/23) modified by fitting a side-tube, approximately 8 mm bore, as shown in Fig. 1 (*a*). A glass helix (Quickfit & Quartz Ex13/25) and a plug of glass-wool serve as a retaining support at the bottom of the column. A condenser at the top and a 100-ml flask at the bottom complete the apparatus.

A weighed amount of material is placed in the column, and solvent is added. When heat is applied, the solvent in the flask is vaporised, passes up the side-arm, is condensed and percolates through the material back into the flask. If the fractionating column is lagged with asbestos cord, but not the side-arm, then the grain is not heated by the solvent vapours to the same extent.

Spectrophotometer or absorptiometer—To measure optical densities at a wavelength of, or centred on, 600 mμ.

REAGENTS—

When applicable, all materials must be of analytical-reagent grade.

Light petroleum, boiling range 60° to 80° C, or n-hexane—Free from aromatic hydrocarbons. The reagent used must be further purified by one of the methods described in Appendix II.

Celite 545—Obtainable from Johns-Manville Co. Ltd., 20, Albert Embankment, London, S.E.11.

Acetone.

Sodium sulphate, anhydrous.

Sulphuric acid mixture—Mix equal volumes of concentrated sulphuric acid and fuming sulphuric acid containing 20 per cent. of sulphur trioxide.

Silica gel—Use material passing a 100-mesh, but retained by a 200-mesh, B.S. sieve. Activate by heating at 250° C for 2 or 3 hours before use.

Diethyl ether.

iso*Amyl alcohol* or n-*amyl alcohol.*

Propylene glycol—Redistil, and reject the first 10 per cent. of the distillate.

Nitration acid—Mix equal volumes of fuming nitric acid and concentrated sulphuric acid. Prepare freshly as required.

Sodium hydroxide solution, 5 per cent. w/v, aqueous.

Sodium chloride solution, saturated, aqueous.

Ethanolic potassium hydroxide solution—Heat 5 g of potassium hydroxide under reflux with 100 ml of absolute ethanol until dissolved. Prepare this solution freshly, and filter immediately before use.

Alternatively, dissolve 5 g of potassium hydroxide and 2 g of urea by heating under reflux with 100 ml of absolute ethanol. Cool, filter, and store in a well stoppered bottle. A slight sediment may gradually develop, and care should be taken not to disturb this when withdrawing portions of the solution for use. The urea acts as a preservative of the solution, which otherwise gradually turns yellow on storage.

Extraction solvent—Mix 2 volumes of light petroleum, boiling range 40° to 60° C, with 1 volume of benzene.

EXTRACTION OF DDT—

Cereals—Extract 25 g of ground cereal in a percolator of the type shown in Fig. 1 (*a*) or in a Soxhlet extractor with *n*-hexane or light petroleum for at least 4 hours, with the solvent boiling briskly under reflux; it is desirable to interrupt the extraction after 2 or 3 hours in order to re-pack the extraction thimble or percolator. Reduce the volume of extract to about 100 ml by gentle distillation or by drawing a current of dry air over its surface at about 40° C. Continue as described under "Clean-up Procedure," but use 10 ml of sulphuric acid mixture instead of 40 ml.

Vegetable oils—Dissolve a 25-ml sample in 100 ml of *n*-hexane in a 500-ml flask, and continue as described under "Clean-up Procedure."

Animal fat—Extract a minced 25-g sample with 150 ml of *n*-hexane in a Soxhlet extractor for at least 1½ hours. Continue as described under "Clean-up Procedure."

Butter—Warm 25 g of butter and 50 ml of *n*-hexane in a 100-ml beaker until solution is complete. Cool the solution, and decant the *n*-hexane layer from the water and curds through a 2-inch column of anhydrous sodium sulphate. Wash the aqueous layer and the sodium sulphate with three 15-ml portions of *n*-hexane, combine the washings and the main *n*-hexane solution in a 500-ml flask, and continue as described under "Clean-up Procedure."

Milk—To 100 ml of chilled milk in a 500-ml flask, slowly add 50 ml of concentrated sulphuric acid, with swirling and cooling. Transfer the mixture to a 250-ml separating funnel, rinse the flask with two 50-ml portions of *n*-hexane, and add the rinsings to the contents of the separating funnel. Add 5 ml of amyl alcohol, and shake vigorously for 1 minute. When the layers have separated (usually after about 30 minutes), run off and reject the aqueous layer, and rinse the *n*-hexane extract into the original flask with hexane. Continue as described under "Clean-up Procedure," but use 10 ml of concentrated sulphuric acid instead of 40 ml of sulphuric acid mixture.

Vegetable material—In a 500-ml flask containing 100 g of minced material place 100 ml each of acetone and *n*-hexane. Rotate the tightly stoppered flask for 1 hour in a mechanical tumbling device. Filter the liquid through a coarse filter-paper into a 500-ml separating funnel, and remove acetone by extracting with three 200-ml portions of water. Dry the

n-hexane extract over anhydrous sodium sulphate, decant into a measuring cylinder, and note the volume of extract. (It is assumed that all the DDT passes into the *n*-hexane layer, and the final result should be corrected according to the volume of extract obtained at this point.) Transfer the extract to a 500-ml flask, and rinse the cylinder several times with *n*-hexane to make up to a total volume of approximately 100 ml. Continue as described under "Clean-up Procedure," but use 10 ml of concentrated sulphuric acid instead of 40 ml of sulphuric acid mixture; also, use concentrated sulphuric acid instead of sulphuric acid mixture in the preparation of the acid - Celite 545 column.

CLEAN-UP PROCEDURE—

Place 4 g of Celite 545 in the chromatographic tube, add 8 g of Celite 545 thoroughly mixed with 4 ml of sulphuric acid mixture, pack firmly and uniformly, and finally add a 1-cm layer of anhydrous sodium sulphate. Fill with *n*-hexane, and allow to drain. To the extract obtained as described under "Extraction of DDT" add 40 ml, unless otherwise indicated, of sulphuric acid mixture in several portions, and swirl the contents of the flask vigorously for about 30 seconds after each addition. If much heat is generated, allow suitable intervals for cooling after each addition of acid mixture. After the final addition of acid mixture, set the flask aside for not less than 30 minutes to allow separation, and then decant the organic layer into the prepared chromatographic tube. When the column has drained, wash the acid mixture in the flask with three successive 75-ml portions of *n*-hexane. For each washing, swirl the contents of the flask vigorously for about 30 seconds, and, after setting aside for at least 15 minutes to allow separation, decant the organic layer into the chromatographic tube. (If emulsification causes slow separation, it is sometimes advantageous to add one or two 5-ml portions of *n*-amyl or *iso*amyl alcohol to the liquid and, if necessary, to allow the final separation to take place overnight; the determination may be interrupted at this stage.) Collect the combined drainings from the column in the evaporator, and reduce the volume of solvent to a few millilitres by heating on a steam-bath.

Stir 8 g of silica gel into a thin slurry with light petroleum or *n*-hexane, and rinse the slurry with more solvent into the glass tube containing the cotton-wool plug. (The use of 100- to 200-mesh material is based on the finding that material containing "fines" tends to pass DDT from a *n*-hexane solution, with the result that recoveries are low in the subsequent elution with diethyl ether. This auxiliary clean-up procedure has been found to be specially necessary for fatty materials.) Rinse the evaporator with a small amount of light petroleum or *n*-hexane, detach the 25-ml flask, and transfer its contents to the prepared tube with a few millilitres of light petroleum or *n*-hexane. When the silica gel has drained, wash the column with four successive 15-ml portions of light petroleum or *n*-hexane. Reject the washings, and drain the column thoroughly by applying air pressure to the top of the tube. Elute the DDT by washing the column with four successive 15-ml portions of diethyl ether. Add 2 drops of propylene glycol to the ether solution, and reduce the volume to a few millilitres in the evaporator. Remove the remaining solvent at 40° C by means of a current of dry air, and finally stand the flask on a steam-bath for 30 minutes. (It has been found that this procedure helps to reduce the blank value, which, with some commodities, is otherwise high; no loss of DDT is experienced at this stage if propylene glycol is added as described.)

NITRATION AND EXTRACTION OF TETRANITRO-DDT—

To the residue add 2 ml of nitration acid, swirl the mixture, transfer the flask to a steam-bath for 30 minutes, and then cool. (The determination may be interrupted at this stage.) Pour the contents of the flask into a separating funnel containing about 25 ml of water, rinse the flask with about 25 ml of water and then with not more than 10 ml of acetone, and add the rinsings to the contents of the separating funnel. By pipette, add 25 ml of extraction solvent, and shake the stoppered funnel vigorously for 1 minute. Allow to separate, and discard the aqueous layer. Add 10 ml of 5 per cent. w/v sodium hydroxide solution, and shake for 30 seconds. Allow to separate, discard the aqueous layer, and repeat the washing with 10-ml portions of alkali until the washings are virtually colourless (three washings are normally sufficient). Drain off the final alkaline layer, add about 20 ml of saturated sodium chloride solution, shake vigorously, allow to separate, and reject the aqueous layer.

COLOUR DEVELOPMENT AND MEASUREMENT—

By pipette, place a suitable aliquot (containing between 10 and 100 μg of DDT) of the solution from the separating funnel in a weighing bottle or suitable test-tube, and evaporate

to dryness at 40° C by means of a current of dry air. Stand the bottle on a steam-bath for 30 minutes, cool, and dissolve the residue in 1 ml of benzene (added by pipette in such a way as to wash down the sides of the bottle). Add 5 ml of ethanolic potassium hydroxide solution, with swirling to mix, replace the stopper, allow the blue colour to develop for 4 minutes, and measure the optical density of the solution in a 1-cm cell at 600 mμ; use a similar cell filled with water as a standard of zero optical density. If the final colour has a brown or reddish appearance, the determination should be repeated, ensuring that the conditions described are strictly adhered to.

PREPARATION OF STANDARD GRAPH—

Prepare a light petroleum or *n*-hexane solution containing 40 μg of *pp'*-DDT per ml. By pipette, place 1 ml of this solution in a 25-ml flask, add 2 drops of propylene glycol, evaporate the solvent at 40° C by means of a current of dry air, and finally stand the flask on a steam-bath for 30 minutes. Proceed with nitration and extraction as described above. By pipette, take a 5-ml aliquot of the nitrated DDT solution from the separating funnel, and carry out the colour development and measurement. Proceed similarly with further aliquots (up to 10 ml) of the original DDT solution, and plot a graph of optical densities of the aliquots against micrograms of *pp'*-DDT present. An optical density of about 0·120 per 10 μg of *pp'*-DDT should be obtained.

Appendix II

METHODS FOR PURIFICATION OF LIGHT PETROLEUM AND *n*-HEXANE

METHOD A—

To 3 litres of light petroleum or *n*-hexane in a 5-litre flask fitted with a glass stirrer slowly add 150 ml of fuming sulphuric acid containing 20 per cent. of sulphur trioxide, and stir vigorously for 1 hour. Transfer to a 5-litre separating funnel, discard the acid layer, and return the light petroleum to the flask. Repeat until the acid layer remains colourless or almost so (three or four sulphonations are usually required).

Add 100 ml of the fuming sulphuric acid and then 80 ml of nitric acid, sp.gr. 1·42, stir for 1 hour, transfer to the 5-litre separating funnel, and discard the acid layer. Repeat the procedure if the acid layer darkens (twice is usually sufficient). Wash the light petroleum successively with three 400-ml portions of distilled water, once with a 2 per cent. solution of sodium hydrogen carbonate or sodium hydroxide and then once again with distilled water. Dry by treatment with anhydrous sodium sulphate.

Distil the light petroleum with about 10 g of freshly fused calcium chloride, fractionate through any suitable column, and collect the required fraction, rejecting the first and the last 200-ml portions. Fractionation with metallic sodium is not desirable, unless great care is taken to remove from the sodium the oil in which it is usually stored.

METHOD B—

Place 800 g of silica gel that has been sifted through a 30-mesh B.S. sieve in a chromatographic tube 5 cm in bore and approximately 1 metre in length. Pass the light petroleum or *n*-hexane through the column in batches of not more than 2 litres. The column can be re-activated by passing 500 ml of acetone through it after the silica gel has been dried in air and heated at 250° C for 2 hours.

Appendix III

MEMBERSHIP OF THE PANEL

The Panel consisted of M. G. Ashley (co-opted April 29th, 1959), Angela N. Bates (co-opted December 16th, 1958), D. F. H. Button, H. Crossley, E. D. Chilwell, J. Colthurst (resigned April 29th, 1959), H. Egan (co-opted March 3rd, 1959), E. G. Hill (co-opted May 7th, 1958), K. Jeffs (co-opted April 29th, 1959), G. A. Lombard, J. T. Martin (Chairman; resigned March 3rd, 1959), E. J. Miller, G. A. Sergeant (resigned March 3rd, 1959), J. H. Shelton (resigned November 10th, 1959), A. Taylor (Secretary from October 7th, 1958), C. C. Thompson (Secretary; resigned October 7th, 1958), E. E. Turtle (Chairman from March

3rd, 1959), J. Ward (resigned April 29th, 1959), R. Wood (co-opted March 3rd, 1959; resigned November 10th, 1959) and C. H. Wordsworth.

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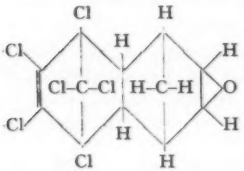
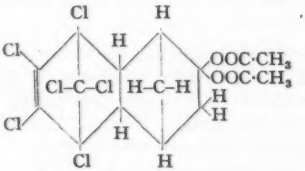
Notes

THE MICRO-DETERMINATION OF ENDRIN

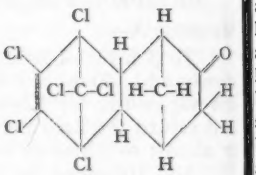
Two colorimetric methods for determining endrin[1] are at present available, the phenyl azide method[2] and the boron trifluoride method.[3] It was hoped that the use of the inconvenient reagents in these methods could be avoided by applying a colorimetric technique recently developed for dieldrin.[4] In this procedure, dieldrin, **I**, was converted by acetic anhydride and catalytic amounts of sulphuric acid to a *gem*-diacetate, **II**, which, when treated with an acidic solution of 2:4-dinitrophenylhydrazine, underwent simultaneous hydrolysis and reaction with the reagent to yield the 2:4-dinitrophenylhydrazone of the ketone, **III**.



(I)



(II)



(III)

It was expected that endrin, a stereoisomer of dieldrin, would follow a similar reaction route. However, with endrin, the reaction product appeared to contain a small amount of free ketone corresponding to **III** (or a readily hydrolysed derivative), the remainder being a diacetate corresponding to **II**. It was assumed that the mixture resulted from a hydrogen ion catalysed isomerisation to the ketone, which underwent simultaneous acetylation by the acetic anhydride present. The diacetate so derived from endrin was much more resistant to hydrolysis than was that from dieldrin. It was considered that if the acetic anhydride was replaced by acetic acid, the reaction would be arrested at the ketone stage, thereby avoiding formation of the stable diacetate.

Experimental

When the method for dieldrin was applied directly to 100 μg of endrin, recovery was only 18 per cent. Paper-chromatographic examination of the mixtures resulting from attempts to prepare the 2:4-dinitrophenylhydrazone in the final stages of the method indicated the presence of substantial amounts of the diacetate, **II**. When samples of the diacetate were heated with the 2:4-dinitrophenylhydrazine reagent solution for 4 hours at 60° C, they yielded only 3·5 per cent. of the 2:4-dinitrophenylhydrazone corresponding to the ketone, **III**. When heated with a 5 per cent. ethanolic solution of potassium hydroxide for 3 hours at 75° C and then with 2:4-dinitrophenylhydrazine for 30 minutes, the yield of 2:4-dinitrophenylhydrazone was only 12 per cent.

From these experiments it appeared that the ketone was formed as an acid-catalysed intermediate in the formation of the diacetate, and the catalytic action of several acids having high dissociation constants on organic solutions of endrin was therefore examined. It was found that 1 μl of sulphuric acid in 1 ml of glacial acetic acid, 90 per cent. acetic acid, 50 per cent. trichloroacetic acid, 1:1-dimethylpropionic acid or ethanol, or 2 mg of *p*-toluenesulphonic acid in 1 ml of di*iso*propyl ether, gave similar conversions of endrin to a ketone. The mixture of acetic and sulphuric acids was preferred, however, owing to the ease with which the acetic acid could be purified. When this reagent was used, the relationship between optical density and amount of

endrin present was found to be $A = 1{\cdot}40 \times 10^{-3} B$, where A is the optical density measured at 440 mμ in a 1-cm cell and B is the number of micrograms of endrin present.

Method

Reagents—

Benzene—Heat analytical-reagent grade benzene under gentle reflux for 8 to 16 hours with 1 g of anhydrous aluminium chloride per litre. Distil, and wash the distillate twice with 20 ml of 10 *N* sodium hydroxide per litre. Heat the washed distillate under reflux for 8 hours with 1 g each of trichloroacetic acid and 2 : 4-dinitrophenylhydrazine per litre, with use of a Dean and Stark trap. Redistil, heat under reflux for 8 to 16 hours with 0·2 g of sodium - potassium alloy per litre, and fractionally redistil through a Widmer column (use a reflux ratio of 1 to 1).

Acetic acid—Prepare as described previously,[4] but heat under reflux for only 8 hours.

Acetic acid - sulphuric acid reagent solution—Cool 10 ml of glacial acetic acid, add 0·1 ml of concentrated sulphuric acid, and add 1 ml of the mixture to 9 ml of glacial acetic acid.

Ethanol—Heat ethanol under reflux for 8 hours with 1 g each of 2 : 4-dinitrophenylhydrazine and trichloroacetic acid, and then fractionally distil through a Widmer column.

2 : 4-Dinitrophenylhydrazine reagent solution—Prepare as described previously.[4]

Tetraethylammonium hydroxide reagent solution—Add 1 ml of 25 per cent. w/v tetraethylammonium hydroxide solution to 24 ml of ethanol. Prepare this solution freshly each day.

Active carbon—Prepare as described previously.[4]

Procedure—

Evaporate a benzene solution containing not more than 200 μg of endrin to dryness at 60° C and 15 to 20 cm pressure. Cool, add 1 ml of acetic acid - sulphuric acid reagent solution, and heat in a water bath for 30 minutes at 60° C. Transfer to a separating funnel with 5 ml of benzene and 5 ml of water, add 2 ml of 10 *N* sodium hydroxide, and shake vigorously. Discard the aqueous layer, wash the benzene layer with 15 ml of water, again discard the aqueous layer, and filter the benzene solution through a 15-mm layer of anhydrous sodium sulphate. Wash the sodium sulphate with a little benzene, add the washings to the main solution, and remove the benzene by evaporation at 60° C and 15 to 20 cm pressure. Dissolve the residue in 0·5 ml of ethanol, and add 0·1 ml of 2 : 4-dinitrophenylhydrazine reagent solution. After 30 minutes, add 5 ml of benzene, and transfer to a separating funnel, rinsing the flask with another 1 ml of benzene. Wash the benzene solution with 10 ml of concentrated hydrochloric acid, 5 ml of 10 *N* sodium hydroxide, 5 ml of concentrated hydrochloric acid and, finally, 15 ml of water. Dry the benzene solution by filtration through a 15-mm layer of anhydrous sodium sulphate into a 10-ml graduated cylinder, and wash the sodium sulphate with benzene until the final volume is 7·5 ml. Add 2·5 ml of tetraethylammonium hydroxide reagent solution, mix thoroughly, and measure the absorption at 440 mμ with a Unicam SP600 spectrophotometer after 1 minute (but not longer than 10 minutes).

Application of the method

The method was applied to the determination of endrin on blackcurrants at the grape stage immediately after spraying. One hundred flower buds were extracted in the cold with three 7-ml portions of benzene. The solution was treated with 0·5 g each of calcium oxide and Darco G carbon and was then poured through a column consisting of 5 g of alumina (Brockmann grade II_2 to III_1[5]) covered by 5 g of anhydrous sodium sulphate in a 15 mm internal diameter chromatographic tube. The amount of endrin found corresponded to 2·04 μg per flower bud, and the recovery of 200 μg of endrin added to an extract of 100 unsprayed flower buds was 91 per cent. However, the blank value, which corresponded to 0·42 μg of endrin per flower bud, was unsatisfactory, and work to decrease interference from plant material is in progress.

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RAPID TITRATION OF CALCIUM IN TRICALCIUM PHOSPHATE WITH EDTA

It is well known that calcium cannot satisfactorily be titrated with ethylenediaminetetra-acetic acid (EDTA) in the presence of phosphate. Cimerman, Alon and Mashall[1] were able to titrate 10 mg of calcium in the presence of up to 60 mg of phosphorus pentoxide by using as titrant a solution containing the sodium and zinc salts of EDTA. We required a method for determining about 80 mg of calcium in the presence of up to 280 mg of phosphorus pentoxide.

Preliminary experiments were made on prepared solutions containing 50 mg of calcium and 344 mg of phosphorus pentoxide (a calcium to phosphorus ratio of 1 to 3). We found that, in solutions suitably buffered with a mixture of ammonium hydroxide and chloride, it was possible to add an excess of EDTA and then to titrate the excess with zinc chloride solution; the end-point was sharp and sensitive to 1 drop of titrant. The recovery of added calcium was the same whether or not phosphorus was present.

Method

Reagents—

Hydrochloric acid, dilute (1 + 4).

EDTA solution, approximately 0·1 N—Dissolve 18·6 g of disodium ethylenediaminetetra-acetate in water, and dilute to 1 litre.

Calcium solution, 0·1 N—Weigh exactly 5·0045 g of analytical-reagent grade calcium carbonate, previously dried in an oven at 100° C, suspend in a little water, dissolve by adding the minimum amount of dilute hydrochloric acid (1 + 4), and dilute to 1 litre.

Zinc solution, approximately 0·1 N—Dissolve approximately 4·07 g of analytical-reagent grade zinc oxide in the minimum amount of hydrochloric acid, and dilute to 1 litre.

Eriochrome black T indicator solution—Dissolve 0·2 g of Eriochrome black T in a mixture of 5 ml of 95 per cent. ethanol and 15 ml of triethanolamine. This solution is stable for several weeks.

Ammonia solution, sp.gr. 0·880.

Procedure—

Accurately weigh about 2 g of tricalcium phosphate into a 250-ml beaker provided with a clock-glass, add 25 ml of dilute hydrochloric acid (1 + 4), and boil for 1 minute. Cool, and dilute with distilled water to 250 ml in a calibrated flask. By pipette, place 25 ml of this solution in a 600-ml beaker, and add 15 ml of dilute hydrochloric acid (1 + 4). Dilute to approximately 250 ml, and add exactly 50 ml of EDTA solution, 10 ml of ammonia solution, sp.gr. 0·880, and 3 drops of indicator solution (in that order). Titrate the excess of EDTA solution with standardised zinc solution (see below) to the first tinge of mauve; the end-point is sharp and permanent.

Carry out a blank titration with each set of samples, the procedure being the same as that described above except for the omission of the sample.

For each sample, note the difference between the volume of zinc solution used in the blank titration and that used in titrating the sample solution.

1 ml 0·1 *N* zinc solution ≡ 0·002004 g of Ca ≡ 0·002804 g of CaO.

Standardisation of zinc solution—

In one of two 600-ml beakers place exactly 25 ml of 0·1 *N* calcium solution. Place 15 ml of dilute hydrochloric acid (1 + 4) in each beaker, and continue as described above, beginning at "Dilute to approximately 250 ml. . . ." Calculate the normality of the zinc solution from the difference between the titres of zinc solution for the contents of the two beakers.

Results

Ten samples of tricalcium phosphate were each tested in triplicate by the proposed method and by the same operator. Replicate tests were carried out on different days with different standardised zinc solutions. Each sample was also analysed gravimetrically by double precipitation of the oxalate and ignition at 500° C to calcium carbonate. The results are shown in Table I.

Statistical examination of these results showed (*a*) that the repeatability of the proposed method was within ±0·25 per cent. of calcium oxide (95 per cent. confidence limits) and (*b*) that results for calcium oxide by the proposed method were, on average, 0·26 per cent. higher than those

by the gravimetric method. For any one determination, the difference between the result by the proposed method and that found gravimetrically will vary between +0·56 and −0·04 per cent., as CaO (95 per cent. confidence limits).

TABLE I

CALCIUM OXIDE FOUND IN TRICALCIUM PHOSPHATE

Sample No.	Calcium oxide found by—proposed method, %	Calcium oxide found by—gravimetric method, %
1	52·2, 52·3, 52·3	51·9
2	51·9, 52·1, 52·0	51·7
3	51·9, 52·0, 52·0	51·7
4	51·8, 51·9, 51·9	51·8
5	51·7, 51·9, 51·8	51·5
6	52·2, 52·5, 52·5	52·2
7	51·7, 51·9, 52·0	51·8
8	51·7, 51·9, 52·0	51·5
9	51·9, 52·1, 52·1	51·7
10	51·9, 52·0, 52·2	51·7

We thank Mr. S. F. Holder for his interest in the work and the Directors of Albright & Wilson (Mfg) Ltd. for permission to publish this Note.

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ALBRIGHT & WILSON (MFG) LTD.
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Apparatus

AN ELECTROMAGNETIC BURETTE VALVE WITH TWO RATES OF DELIVERY

MECHANICALLY-OPERATED burettes fall into four main classes, in the simplest of which the flow of liquid is governed by compression of a flexible outlet tube.[1] Control by this method is satisfactory for most aqueous solutions, and the ready replacement of rubber or plastic tubing allows a compression-type valve to be used with certain organic solvents.[2] Most gravity-fed mechanical burettes have a single pre-set rate of delivery that may lead to undue slowness or end-point overshoot in automatic titrimetry. Rapid delivery of most of the titrant and then a dropwise approach to the end-point may be obtained by using two independent valves arranged in parallel. The more satisfactory arrangement shown in Fig. 1 utilises a single compression-type control with two adjustable degrees of opening.

The mechanism is mounted in a shallow box, the sides of which are of 1½-inch × ¼-inch hardwood strip. Most of the parts are mounted on a back of $\frac{3}{16}$-inch thick transparent Perspex sheet; a similar sheet ⅛ inch thick forms a front cover that can be readily removed for adjustment purposes. A 6-inch length of ½-inch diameter brass rod, A, is screwed to the top of the box. This allows the entire unit to be supported in a screw clamp on the burette stand. Outlet B is of cycle-valve tubing and connects the burette proper to glass tubing jet C. The latter is supported in two small Terry clips and is inserted through a hole in the side of the box. Two pairs of small pins, driven into hardwood pressure block D (see Fig. 1 (*b*)), locate the outlet tubing squarely beneath pressure lever E. This and fast-flow lever F are ½-inch × ⅛-inch mild-steel strip, which is twisted as shown. The same material is used for the cores of electromagnets G and H. The latter are wound to suit the desired operating voltage; typically, about 150 feet of No. 24 s.w.g. wire for each magnet is suitable for a 4- to 6-volt supply. Each core is backed with a strip of $\frac{1}{16}$-inch thick brass or aluminium, the ends being bent upwards to form cheeks to secure the windings. The magnets are held between ¼-inch wide brass retaining strips by screws that pass through distance pieces and then through the back.

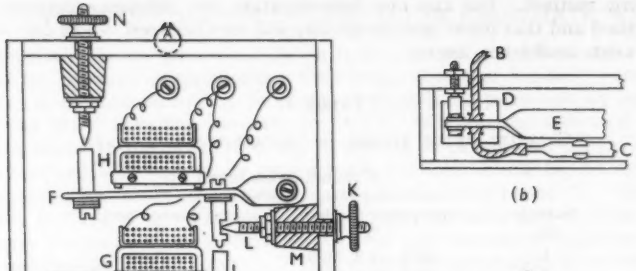

Fig. 1. (*a*) Front view of electromagnetic burette valve; (*b*) plan view of pressure block; (*c*) alternative form of adjustment

When electromagnet G is energised, the pressure lever rises until brass pillar I encounters brass stop J. Slow flow of titrant then occurs. Energisation of both magnets permits rapid flow, since stop J is then lifted to allow the pressure lever to rise higher. The rate of "slow flow" may be progressively reduced from a continuous stream to one drop in several seconds by tightening thumbnut K. This retracts screw L and lowers stop J. The screw is a tight fit in soft rubber stopper M, which is compressed when K is tightened. This form of adjustment is insensitive to vibration.[3] The alternative form (see Fig. 1 (*c*)) affords a wider range of adjustment. In place of a stopper, a short cylinder of rubber sponge surmounted by a small plastic reagent-bottle cap is used. "Fast flow" is adjusted by thumbnut N, which limits the extent to which stop J is lifted when both magnets are energised.

The correct loading of the levers is obtained by the addition of washers to the screws which retain the pillars. The need for this adjustment is minimised if a 25-ohm variable resistor is placed in series with electromagnet G.

This apparatus was developed with the partial support of the U.S. Atomic Energy Commission.

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Book Reviews

HANDBUCH DER PAPIERCHROMATOGRAPHIE. Edited by I. M. HAIS and K. MACEK. Band I. Grundlagen und Technik. Pp. xxvi + 860. Jena, Germany: Veb Gustav Fischer Verlag. 1958. Price DM 58.40.

This is the German edition of the Handbook of Paper Chromatography under the editorship of Hais and Macek with sections written by experts in the Universities, Research Institutes and Industry of Czechoslovakia. It was originally published in 1954, but a comparison of the two editions indicates that this book is vastly better produced not only from the point of view of printing and illustrations (many are in colour), but also from the theoretical and practical aspects. The literature has been well covered up to about 1956, but there are few if any references to anything later. This has probably not the disadvantages that might appear at first sight, because, from the point of view of much chromatography, most of the fundamental developments had been made by that time; the number of applications since 1956 has, however, been legion.

The general part of the book, which occupies some 200 pages, presents a good introduction to the principles of chromatography and will repay careful study not only by the beginner, but also by those who have practised the technique for so long that some aspects of it may have been overlooked. The reviewer, for example, was interested to be reminded of the correlation that workers in quite different fields from his own had made on the relationship between structure and the chromatographic properties of substances. Although these references were mainly to organic compounds, such correlations are now being widely used in the chromatography of inorganic anions, such as phosphates.

The major part of the introduction is a very full consideration of the practical aspects of the techniques of chromatography and is extremely well illustrated. The excellent descriptions of the methods employed and the difficulties that can be experienced is a notable feature of the book. It would be difficult to find any really important omissions, though there is no mention of phosphorylated paper, which is of current interest, but this is to be expected since the book is dated about 1956. Besides the details of qualitative separations and the methods of final estimation for quantitative determinations, there is a useful chapter on preparative methods, including those for continuous chromatography.

Almost half of the book is devoted to special separations and covers all types of organic and biological problems; it is arranged under the headings—alcohols, aldehydes and ketones, aliphatic acids, carbohydrates, phenols and aromatic acids, steroids, hydrocarbons, aliphatic and aromatic amines, nitro-compounds, amino acids, peptides, proteins, purine, pyrimidine and components of nucleic acids, alkaloids, nitrogen-containing heterocyclics, organic sulphur compounds, vitamins, antibiotics, insecticides, synthetic dyestuffs and inorganic substances. The completeness of the book is well indicated by this list and further by some 60 pages with experimental notes on the composition of spray reagents and details of important quantitative determinations.

This is a handbook that every librarian will find to be frequently consulted and every chromatographer will want to possess. The editors and publishers are to be congratulated on its excellent production.

F. H. POLLARD

TRACE TECHNIQUES USING THE K1000 CATHODE RAY POLAROGRAPH. Volume I. By J. HETMAN, F.R.I.C. Pp. 48. Camberley, Surrey: Southern Instruments Ltd. 1959. Price 25s.

The purpose of this book is to draw attention to new methods for use with a particular polarographic instrument. Thirty methods are described and they cover a large range of subjects, *e.g.*, the determination of nitrobenzene in aniline, of vitamin C in orange juice, of copper, lead and zinc in lubricating oil, of sodium and potassium in tap-water and of uranium in monazites.

There is a great deal in this book that will worry the analyst, *e.g*, the determinations are stated to deal with traces, but the only example of the simultaneous determination of fumaric and maleic acids deals with an equimolecular mixture. Moreover, if the analyst follows the directions given for the simultaneous determinations of copper, lead and iron in phosphoric acid he will finish up with a semi-solid mixture, and polarography will be difficult, to say the least.

Then again, it will be a very salutary experience for the analyst who uses the method for determining copper, lead, cadmium and zinc in P.V.C. "plastics" if he compares "added" and "found" in his results.

There is much in this book that can be criticised along these lines. In the reviewer's opinion it is a good idea to bring forward a book that will inform the analyst what can be accomplished with a particular polarographic instrument. Nevertheless, it is to be hoped that if further editions are to be published the services of a professional analyst will be enlisted. He would make quite certain that the limitations of the various methods were thoroughly described.

J. HASLAM

OXOSTEROIDS. THE USE OF PHENOLIC HYDRAZIDES FOR DETECTION, CHARACTERISATION AND ESTIMATION. By BERNARD CAMBER, M.D. Pp. viii + 79. London: H. K. Lewis & Co. Ltd. 1960. Price 12s. 6d.

This short volume should be of value to all interested in the investigation of steroids containing oxo-groups. Although the reagents the author describes are not as sensitive as some in regular use, their versatility would suggest that they deserve wider application than they have received. The use of 2-hydroxy-3-naphthoylhydrazide, β-resorcyl hydrazide and especially of salicyloyl hydrazide to give fluorescent compounds is described in detail, and the reaction of the salicyl hydrazones of the steroid with *p*-dialkylaniline greatly extends the utility of the method to include oxosteroids that do not themselves produce fluorescence with the reagent. It is useful to have available a collected account of the investigations hitherto discussed by Dr. Camber in only a series of short papers. His work deserves greater consideration than it has received; perhaps this book will help.

C. H. GRAY

AN INTRODUCTION TO PRACTICAL INFRA-RED SPECTROSCOPY. By A. D. CROSS, B.Sc., Ph.D. Pp. viii + 80. London: Butterworths Scientific Publications. 1960. Price 17s. 6d.

Infra-red spectroscopy is widely used by the modern organic chemist to help elucidate the structure of new and unknown substances and to provide a degree of analytical control of complicated syntheses that would have been unthinkable a few years ago. In multi-stage organic syntheses, it has become customary to obtain an infra-red spectrum of the product after each chemical reaction; this spectrum shows how much of the anticipated product has been formed and indicates the nature of the product if the reaction has not gone the right way.

The recording and interpretation of an infra-red spectrum were, until recently, tasks for the specialist, but the introduction of relatively simple, reliable spectrophotometers and the publication of correlation tables listing the absorption-frequency ranges for most organic groups have excited a much wider interest in the technique. It is to satisfy the practical interests of undergraduate and research students that Dr. Cross, a lecturer at Imperial College, has written this introductory manual.

The first half of the book summarises the theory and describes practical aspects of infra-red spectroscopy, such as instrument design and sampling techniques. Most of the sampling procedures in common use are mentioned, but, rather surprisingly, bromoform has been omitted from the list of recommended solvents; bromoform transmits over a wider spectral range and has distinct advantages over chloroform. A table, in which the principal features of double-beam spectrometers currently available in Germany, Japan, Russia, the U.S.A. and the United Kingdom are compared, provides a novel feature.

The second half of the book contains 31 correlation charts and tables, which list the absorption-frequency ranges for various chemical groups and provide the basic information needed for the interpretation of a spectrum. A vast amount of information has been packed into the tables; space limitation has, however, prevented the inclusion of correlation tables for special classes of compounds, such as steroidal sapogenins, and, what is more unfortunate, has prevented a critical discussion of the scope and limitations of the frequency correlations listed in each table. Absorption bands that for molecules of all types fall within a wide frequency range often appear in a much narrower range for one class of compound and are, therefore, particularly valuable for the identification of that class of compound. For information of this sort and for detailed references to the original literature, the chemist must turn to L. J. Bellamy's and to R. N. Jones and C. Sandorfy's well known monographs or to recent reviews of the subject. Undiscriminating use of the correlation tables could, as is pointed out by the author, lead frequently to erroneous conclusions.

The book is free from errors and, with the warning given in the last paragraph, can be recommended to students of organic chemistry and as introductory text to anyone interested in infra-red spectroscopy.

J. E. PAGE

APPLICATIONS OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN ORGANIC CHEMISTRY. By L. M. JACKMAN. Pp. xii + 134. London, New York, Oxford and Paris: Pergamon Press Ltd. 1959. Price 35s.

High-resolution nuclear-magnetic-resonance (NMR) spectroscopy, like ultra-violet and infra-red spectroscopy before it, has developed into a physical method of great power for studying the structures of large and small molecules. The organic literature is already dotted with NMR data quoted for the purpose of confirming the structures of molecules, and it is becoming essential that even the chemist who does not yet have access to the necessary equipment should be able to understand the significance of the results obtained.

Because hydrogen nuclei give strong spectra and because the positions of the resonances of different hydrogen-containing groups depend in a fairly reproducible way on the nature of the chemical environment, it has become possible to draw up lists of band positions (chemical shifts) characteristic of different organic structural groups in much the same way that lists can be made of characteristic infra-red group frequencies. However, the "chemical shifts" are not measurements of the *absolute* magnetic field at which the nuclei give resonance but, for experimental reasons, of their *relative* values with respect to a chosen reference compound, such as water, *cyclo*hexane or silicon tetramethyl. The reference compound may be dissolved in the sample being studied (internal-reference) or enclosed in a separate glass capillary (external reference), and the results obtained depend somewhat on the method used. For the purposes of making structural correlations the internal-reference method is to be preferred, but it has not been used consistently in the past; further, different workers have chosen different reference substances, and so it is a tedious business for even the expert to convert, and thereby compare, data from different laboratories.

The particular merit of this book is that it collects together most of the organic structural correlations and quotes these systematically and uniformly on the same scale of reference, *viz.*, with silicon tetramethyl as an internal standard. The suggestion for this reference substance (which is chosen because its resonance falls conveniently just outside the range for most organic groupings) was made by G. V. D. Tiers 2 years ago, and most of the experimental data also came from his unpublished work. This scale of reference is becoming increasingly used, and in the opinion of the reviewer the author of this book has made a wise choice in sticking to this scale. If the availability of these systematic data persuades most laboratories to adopt this scale, the book could also perform a major service to NMR spectroscopy as a whole.

In addition to giving a very good discussion of the characteristic chemical shift data the book gives a clear account of the theoretical factors causing the observed pattern of chemical shifts, of the additional (often vitally important) information to be derived from the fine-structure of resonances resulting from interaction between neighbouring nuclei and of the main experimental procedures. The last two chapters discuss the interpretation of the spectra of complex molecules, and applications of this method to evaluating stereochemical factors.

The book is reasonably priced and has been rapidly produced so that it is surprisingly up-to-date—although a few typographical errors (and the misorientation of Figure 3.12) have presumably resulted from the speed. It can, however, be strongly recommended to all interested in the qualitative (and sometimes quantitative) analysis of the structure of organic molecules.

N. SHEPPARD

BRITISH VETERINARY CODEX 1953: SUPPLEMENT 1959. Published by direction of the Council of the Pharmaceutical Society of Great Britain. Pp. xviii + 134. London: The Pharmaceutical Press. 1959. Price 35s.

This Supplement is partly concerned with changes in descriptions and standards necessitated by the revisions and additions embodied in the British Pharmacopoeia 1958 and the British Pharmaceutical Codex 1959, since many of the drugs and preparations included in the British Veterinary Codex 1953 (reviewed in *Analyst*, 1954, **79**, 462) are described by reference to one or other of these publications. Apart from these formalities there are 47 new monographs in Part I on newly introduced drugs, and the standards include details of purity tests and assays or, when appropriate, reference to the B.P. or B.P.C. It is of interest to note that for the trypanocide dimidium bromide the analytical standard has been re-drafted and the assay changed from a silver titration to a special procedure for determining the total nitrogen.

There are a number of new monographs covering Part II of the B. Vet. C. (antisera, vaccines, etc.) and also additions to the Formulary (Part III), including an interesting, but somewhat brief, section on Antibiotics as Dietary Supplements.

N. L. ALLPORT

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 39. Editor-in-Chief: MAX TISHLER. Pp. viii + 114. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1959. Price $4.00; 32s.

This number is the last of the decad that will doubtless appear in due time as Collective Vol. 4. It describes the preparations of—methylenecyclohexane, 9-methylfluorene and triptycene (9:10-dihydro-9:10-*o*-phenyleneanthracene); 1-phenylpent-1-en-4-yn-3-ol; 3:3-diethoxy-1-phenyl-propyne; 3:4-diacetylhexane-2:5-dione and 2:4:4-trimethylcyclopentanone; methyl cyclopentanecarboxylate; β-methyl-β-phenylglutaric acid; 9:10-dihydroxystearic acid (low-melting isomer); di(carboxymethyl) trithiocarbonate; *N*-cyclohexylmethyldimethylamine; tetracyanoethylene, ααβ-triphenylpropionitrile, ethyl α-cyano-β-methylcinnamate, 2-dicyanomethylene-1:3-dioxolan, *NN*-dimethyl-*p*-tricyanovinylaniline and αα′-dicyano-β-methyl-β-phenylglutarimide; *NN*-dimethylhydroxyammonium chloride; triphenylboroxine and di-*n*-butyldivinyltin; 3-methylfuran, 3-methyl-2-furoic acid and its methyl ester, 1:4-dithian, 2:5-diamino-3:4-dicyanothiophen, 3-*p*-acetamidophenylrhodanine, indazole, indole-3-aldehyde, 5-cyano-4-hydroxy- and 4-amino-5-ethoxycarbonyl-2-mercaptopyrimidine.

B. A. ELLIS

THE CHEMICAL ANALYSIS OF AIR POLLUTANTS. By MORRIS B. JACOBS, Ph.D. Pp. xviii + 430. New York and London: Interscience Publishers Inc. 1960. Price $13.50; 102s.

It seems that Dr. Morris B. Jacobs is a versatile author. His book on the Chemical Analysis of Foods and Food Products is known in this country, and he is co-author of a monograph on Chemical Methods in Industrial Hygiene and has published another monograph on Analytical Chemistry of Industrial Poisons. This book is No. 10 in a series on Applied Analytical Chemistry, and it deals with the Chemical Analysis of Air Pollutants.

To the extent that it is a collection of well documented abstracts of publications (mainly American) on the subject of the examination of impurities in air, the book is useful for reference, although the reader may well be irritated by too-frequent reminders that important aspects of the subject he is studying have been omitted here because they have been "dealt with in detail" by the author in other text-books. Dr. Jacobs, however, has not been content only with abstracts and has included a variety of working directions for determining specific and non-specific air pollutants. Anyone who attempts to follow some of these directions from the details given in the text would be liable to find himself in serious difficulty. A few of the defects may be due to careless proof-reading, although failure to recognise them as compositor's errors might have unfortunate consequences. But there are also more fundamental misdirections, which can only be attributed to a somewhat uncritical approach on the part of the author himself. Not everyone who occasionally engages in the investigation of air pollution has the experience necessary to recognise a faulty analytical direction or technique on sight, and for this reason the book cannot be enthusiastically recommended as a reliable guide to analysis.

In this country, the routine examination of rain water from deposit gauges and the estimation of sulphur dioxide pollution of air are invariably carried out by methods standardised by the D.S.I.R. Nevertheless, problems of identifying or estimating, or both, some specific industrial contamination frequently occur, and their solution is often an essential preliminary to litigation. In such circumstances, Dr. Jacobs's book will be useful for reference and will give some general guidance to those without previous experience.

J. G. SHERRATT

ORGANIC REACTIONS. Volume X. Editor-in-Chief: ROGER ADAMS. Pp. viii + 563. New York: John Wiley & Sons Inc.; London: Chapman and Hall Ltd. 1959. Price $12.00; 96s.

The most obvious difference between this volume and its predecessors in this invaluable series is the fact that it contains only three chapters, as compared with an average of nine in the earlier volumes. This represents a further stage in what appears to be a policy of dealing with some of the long-established standard reactions of organic chemistry, which necessarily have a voluminous literature, and is most welcome.

More than two-thirds of the volume is taken up by an article on the Michael reaction, by E. D. Bergmann, D. Ginsburg and R. Pappo, which runs to 377 pages, cites 1045 references and is a complete monograph on the subject. In accordance with the general character of the series, the emphasis is on the preparative aspects of the reaction, but mechanism has not been neglected, and a good and clear account of present views on mechanism is included; in this connection it

is rather surprising to find that the leading references given for the reversibility of the reaction are to papers published during the 1950's, since reversibility was observed by Vorländer in 1900 and studied by Ingold and his co-workers in the 1920's. This, however, is but a minor blemish in a most excellent and authoritative review.

The other two chapters, which deal with the coupling of diazonium salts with aliphatic carbon atoms (by S. M. Parmenter; 142 pages; 480 references) and with the Japp - Klingemann reaction (by R. R. Phillips; 36 pages; 118 references), are concerned, of course, with very closely related topics. Both are of the usual type and both are welcome reviews of useful reactions; it is unfortunate that, in the latter article, one of the examples chosen to illustrate the cyclisation of a Japp - Klingemann product to an indole has been shown to be incorrect.

In all three articles the literature is fully covered only up to the end of 1955, although the chapter on the Michael reaction contains some references to papers published in 1957. Although not so important in a work of this kind as in others of less permanent value, it can only be regretted that publishing delays can render books of this kind 4 years out of date when they appear.

Although this volume is 100 pages longer than volume 9 and, if anything, even better produced, it is encouraging to find that it costs no more. Like its predecessor, this volume is very good value, and all practising organic chemists will wish to possess a copy.

H. N. RYDON

HENRY CAVENDISH: HIS LIFE AND SCIENTIFIC WORK. By A. J. BERRY, M.A. Pp. 208. London: Hutchinson & Co. (Publishers) Ltd. 1960. Price 35s.

All readers will know of the famous experiments of Cavendish on the composition of water, but the wide range of his interests may not be so familiar. Besides his published work, Cavendish left a good deal of manuscript material, some of it describing experiments of very great importance. His published and manuscript work has been collected and published in two large volumes, but there has been no recent study of Cavendish of readable size. Mr. Berry's book provides a clear and interesting survey of what is known of the life of Cavendish and of his chemical and physical researches. It gives a short preliminary survey of the state of science in his time.

The chapter on Cavendish's chemical work shows that he was the first to characterise hydrogen and carbon dioxide, both known before but not carefully investigated. His paper on Rathbone-Place water (1767) really laid the foundations of water analysis. The experiments on the composition of water are fully described. Cavendish was the first to establish the composition of nitric acid, and in the work he noticed what must have been argon. His experiments on the freezing-points of mixtures of nitric acid and water showed maxima corresponding to compounds and also eutectics.

Cavendish made many experiments on heat. His electrical researches were of the first importance, both the published (establishing the inverse-square law) and unpublished (including the conductivity of salt solutions). What is generally considered to be his most celebrated paper, on the mean density of the earth (1798), is a masterpiece of experimental skill and mathematical treatment. Mr. Berry reviews all this work fully, yet without assuming mathematical knowledge. The book is illustrated by some excellent plates. It is one that can be strongly recommended to the attention of chemists, who will find in it much to interest them. As a brief yet compendious survey of the work of one of the greatest English scientists, it deserves the highest praise. Everything dealt with is made interesting, and the book is a pleasure to read.

J. R. PARTINGTON

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THE RADIOCHEMISTRY OF AMERICIUM AND CURIUM. By R. A. PENNEMAN and T. K. KEENAN. Pp. vi + 62. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 75 cents.
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THE RADIOCHEMISTRY OF RHODIUM. By G. R. CHOPPIN. Pp. vi + 32. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.
Nuclear Science Series: NAS—NS—3008.

THE RADIOCHEMISTRY OF MOLYBDENUM. By E. M. SCADDEN and N. E. BALLOU. Pp. vi + 38. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.
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OPTICAL CRYSTALLOGRAPHY, WITH PARTICULAR REFERENCE TO THE USE AND THEORY OF THE POLARIZING MICROSCOPE. By ERNEST E. WAHLSTROM. Third Edition. Pp. x + 356. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1960. Price $8.50; 68s.

Papers for Publication in *The Analyst*

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